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THE

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OF THE

ROYAL DUBLIN SOCIETY

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1. REPORT OF THE IRISH RADIUM COMMITTEE FOR THE YEAR 1941. Including Reports from OSWALD J. MURPHY, M.B., B.Ch., St. Vincent's Hospital, Dublin; ST. ANNE'S HOSPITAL, DUBLIN (*Radiologist*—Dr. Oliver Chance); R. E. TOTTENHAM, M.D., B.Ch., B.A.O., City and County Hospital, Londonderry; and OLIVER CHANCE, M.B., F.F.R., D.M.R.E., Richmond, Sir Patrick Dun's, Dr. Steevens', Rotunda, and Coombe Hospitals.

2. THE CHEMICAL CONSTITUENTS OF LICHENS FOUND IN IRELAND. *CLADONIA IMPEXA* HARM. By T. W. BREADEN, M.Sc., J. KEANE, PH.D., and T. J. NOLAN, D.Sc., University College, Dublin.

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4. THE IDENTITY OF THE TOP-NECROSIS VIRUS IN UP-TO-DATE POTATO. By PHYLLIS E. M. CLINCH, Albert Agricultural College, Glasnevin, Dublin. (Plate I.)

5. FORMATION OF GYNOSPORE, FEMALE GAMETOPHYTE, AND ARCHEGONIA IN *SEQUOIA*. By W. J. LOOBY and J. DOYLE, Botany Department, University College, Dublin.

[*Authors alone are responsible for all opinions expressed in their Communications.*]

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THE

SCIENTIFIC PROCEEDINGS

OF

THE ROYAL DUBLIN SOCIETY.

No. 1.

REPORT OF THE IRISH RADIUM COMMITTEE FOR THE YEAR 1941.

[Read MAY 19. Published separately JULY 6, 1942.]

1,400 tubes containing 10,613 mc. of radon were issued during 1941, as compared with 1,037 tubes containing 8,571 in 1940.

As the total number of cases treated of which reports have been received is very close to that for 1940, this indicates an increase in the quantity of radon used in each case.

Although the stock of radium has for many years been kept in a fireproof strong room, it was decided towards the end of the year that further protection was desirable. This was effected early in 1942 by enclosing the flasks containing the radium solution in a lead casting weighing some 9 cwt., which in turn is buried in a reinforced concrete block weighing some 13 cwt. This has in no way affected the supply of radon.

Dr. Bernard A. Cousins was elected to the Radiation Exhibition falling vacant during the year.

Returns from the chief users record the treatment of the following cases with radon or radium element during 1941:—

MALIGNANT CASES.

Dr. Oswald Murphy, St. Vincent's Hospital, Dublin ...	90
Dr. Oliver Chance, Richmond, Sir P. Dun's, Dr. Steevens', Rotunda, and Coombe Hospitals, Dublin	17
Dr. d'Abreu, City and County Infirmary, Waterford ...	17
Dr. Edward Solomons, Dr. Steevens' Hospital	2
Dr. R. E. Tottenham, City and County Hospital, Londonderry ...	2
St. Anne's Hospital, Dublin	139
Royal City of Dublin Hospital	39
St. John's Hospital, Limerick	6
National Maternity Hospital, Dublin	2
Total, ...	314

NON-MALIGNANT CASES.

Dr. Oswald Murphy	77
Dr. Oliver Chance	17
Dr. R. E. Tottenham	1
National Maternity Hospital, Dublin	2
St. Anne's Hospital, Dublin	1
Total, ...	98

The returns show that for the cases of malignant disease first seen during 1941 the numbers treated by the different methods were as follows:—

1.	Number of cases of all types of malignant disease seen during 1941 ...	1,204
2.	„ „ treated during 1941	1,015
3.	„ „ „ by Surgery alone	176
4.	„ „ „ by Radium alone	222
5.	„ „ „ by X-ray alone	457
6.	„ „ „ by Surgery and Radium ...	30
7.	„ „ „ by Surgery and X-rays	68
8.	„ „ „ by Radium and X-rays	60
9.	„ „ „ by Surgery, Radium and X-rays	2

Radon treatment was employed in the Royal City of Dublin Hospital from the month of September until the end of the year, as the impossibility of obtaining renewals of apparatus rendered it impossible to continue the X-ray treatment previously employed.

All the cases treated in St. John's Hospital, Limerick, were rodent ulcers in various situations on the face. Clinical cures were effected in all cases.

The two non-malignant cases treated in the National Maternity Hospital yielded satisfactory results.

The following reports have been received from some of the larger users:—

Report by Oswald J. Murphy, M.B., B.Ch.,

St. Vincent's Hospital, Dublin.

The cases covered by this report were mostly treated by needles containing radium salt.

MALIGNANT CASES.

Treated by Surgery and Radium, 10; Radium and X-rays, 4; Radium alone, 76—Total, 90.

	Treated.	No Glands.	Glands.	Improved.	Well.
Carcinoma of Ear ...	4	2	2	3	1
„ of Lip ...	7	5	2	2	5
„ of Cheek ...	2	2	—	1	1
„ of Parotid ...	2	—	—	2	—
„ of Tongue ...	5	3	2	3	2
„ of Bladder ...	2	—	—	1	1
„ Uterine Cervix	7.	Group I, 5.	Group II, 2.		
„ „ Body	6.				

	Treated.	Improved.	Well.
Carcinoma of the Breast (Secondaries) ...	4	3	—
„ of the Larynx (Intrinsic) ...	2	1	1
„ of the Skin	7	3	4
Rodent Ulcers	42	4	38

One case of carcinoma of the uterine body was practically bled white at the time of radium treatment. Owing to unforeseen circumstances she was only operated on 11½ months later. The uterus was normal in size, but very fibrotic. There was no microscopical evidence of malignancy, and there were no adhesions and no signs of spread or secondaries.

NON-MALIGNANT CASES.

T.B. Cervical Glands, 2; T.B. Peritonitis, 14; Nævi, 22; Lupus Vulgaris, 4; Pruritis Vulvae, 1; Warts, 32; Keloids, 2—Total, 77.

Report from St. Anne's Hospital, Dublin.

Radiologist: DR. OLIVER CHANCE.

Below is given an analysis of treatment of malignant cases in 1941.

Number of cases of all types of malignant diseases seen, 1941 ...		659
Number of cases of all types of malignant diseases treated ...		511
Treated by Surgery alone	37	
„ „ Radium alone	89	
„ „ X-rays alone	318	
„ „ Surgery and Radium	6	
„ „ Surgery and X-rays	17	
„ „ Radium and X-rays	43	
„ „ Surgery, Radium and X-rays ...	1	
	511	

Of the patients treated with radium, radon was used on 56 occasions in the treatment of 38 patients, all of whom were suffering from cancer of the cervix uteri. The results obtained in these cases were as follows:—

Stage.	Total treated.	Alive.	Dead.	Untraced.
1	9	4	2	3
2	10	9	1	0
3	18	12	2	4
Post-operative recurrence ...	1	1		

Of the benign patients treated during the year, in only one case was radon used. This was a child suffering from a nævus. The child was still under treatment.

Report by R. E. Tottenham, M.D., B.Ch., B.A.O.,
City and County Hospital, Londonderry.

Case 1.—Miss G. was a degenerating myoma of the uterus, inoperable, which I treated with radium alone. The pathological report was myoma becoming sarcomatous. I am afraid I have lost trace of her since.

Case 2 was a carcinoma of the cervical canal. I attempted to do a hysterectomy for her, but the cervix broke across at its middle owing to the presence of the tumour. I decided to treat the stump of the breaking-down cervix with radium in preference to trying to remove it in fragments. She received 2,000 M.E.H., approximately, and then refused further treatment. An interesting point in connection with this case was that the abdominal wound broke down and a very foul-smelling discharge escaped. On applying radium to the stump of the cervix from below, as described, the abdominal discharge lessened materially, and I am of the opinion that the wound healed up much more quickly than it otherwise would have done. (This is purely an opinion.)

Case 3.—The third case was treated by total removal of the uterus.

Perhaps I may add that in one case of Caesarean Section (in which the operation was not performed by me) the wound broke down from top to bottom and the patient was in an exceedingly dangerous condition with temperature, pulse, etc. More or less in desperation I applied some radon to the abdomen in very small doses, insulated by brass, and moved every hour by the nurse. The radon was in needles strapped on the outside of a brass plate, 2 mm. thick or more, which was about 8″ by 2″ long, and the six needles were equally distributed along the plate, the whole plate being moved on each occasion. The total application was only for 12 hours. The patient improved remarkably —whether it was due to the action of the radium or a coincidence I cannot say, but the improvement occurred. I, of course, had to take the risk of damage to the ovaries, but as there was profound abdominal sepsis the chances of the patient having another child were not very great at the best of times.

P.S.—I have not included cases of ovarian tumours becoming malignant; because they are not generally applicable to radium therapy.

Report by Oliver Chance, M.B., F.F.R., D.M.R.E.,
Richmond, Sir P. Dun's, Dr. Steevens', Rotunda, and Coombe Hospitals.

Owing to war conditions, the year 1941 was an exceedingly difficult one. Shortage of transport caused a large proportion of the patients to delay in seeking medical advice, and rendered the adequate following up of these patients impossible. Below is given a summary of the patients treated during the year, and an analysis of the methods of treatment used. The increase in the number of cases treated by X-rays, referred to in last year's Report, continues. This is, of course, largely due to the fact that contact X-rays have replaced radium in the treatment of most cases of superficial malignant disease.

TOTAL MALIGNANT CASES TREATED BY RADIATION (115).

Disease.	X-rays.	Radium.	X-rays and Surgery.	X-rays and Radium.	Radium and Surgery.	Unsuitable.	Total Treated.
Rodent ulcers ...	30	1	–	–	–	2	31
Carcinoma Skin ...	9	2	1	1	–	1	13
„ Lip ...	11	1	1	–	1	–	14
„ Tongue	3	3	–	–	–	2	6
„ Palate	1	–	2	–	–	–	3
„ Tonsil	2	–	–	–	–	–	2
„ Larynx (Extrinsic)	7	–	–	–	–	–	7
„ Larynx (Intrinsic)	–	2	–	–	–	–	2
„ Postcricoid	3	–	–	–	–	–	3
„ Œsophagus	–	–	1	–	–	2	1
„ Thyroid	–	–	3	–	–	–	3
„ Breast	4	–	7	–	1	1	12
„ Cervix	1	1	1	3	1	1	7
„ Body of Uterus	–	–	1	–	–	1	1
„ Ovary	–	–	1	–	–	–	1
„ Rectum	–	–	–	–	–	1	0
„ Kidney	–	–	2	–	–	–	2
„ Penis	1	–	–	–	–	–	1
Sarcoma (Various)	3	–	3	–	–	1	6
	75	10	23	4	3	12	115

TOTAL NON-MALIGNANT CASES TREATED BY RADIATION (48).

Disease.	X-rays.	Radium.	X-rays and Surgery.	X-rays and Radium.	Radium and Surgery.	Unsuitable.	Total Treated.
Toxic goitre ...	4	10	–	1	–	–	15
Tubercular peritonitis	–	5	–	–	–	–	5
Tubercular adenitis	4	–	2	–	–	–	6
Benign uterine conditions ...	2	1	–	–	–	–	3
Myelogenous leukæmia	1	–	–	–	–	–	1
Prostatic hypertrophy	2	–	–	–	–	–	2
Nævi	11	–	–	–	–	–	11
Plantar corns and warts ...	5	–	–	–	–	–	5
	29	16	2	1	–	–	48

No. 2.

THE CHEMICAL CONSTITUENTS OF LICHENS FOUND IN IRELAND.

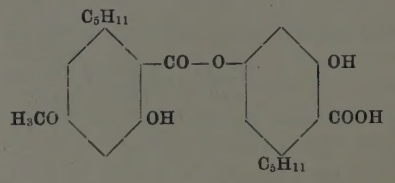
CLADONIA IMPEXA HARM.

By T. W. BREADEN, M.Sc., J. KEANE, Ph.D., and T. J. NOLAN, D.Sc.,
University College, Dublin

[Read May 27, 1941. Published separately July 28, 1942.]

There appears to be no previous record of the occurrence in Ireland of the lichen *Cladonia impexa* Harm. It is not mentioned in the standard reference—Knowles, "The Lichens of Ireland." We have, however, found it growing in considerable quantities on peaty soil, amongst granite rocks on the west side of Killiney Hill, Co. Dublin. The specimens were identified for us by Mr. Mackenzie-Lamb, of the Natural History Section of the British Museum, to whom we wish to express our thanks. Asahina records (Acta Phytochimica, 1934, 8, 52) that the lichen shows a negative reaction to benzidine and p-phenylenediamine, indicating the absence of depsidones of the salazic acid type.

We have found the predominant constituent to be l-usnic acid. We have also isolated perlatolic acid



previously found by Asahina and Fuzikawa (Ber., 1935, 68, 634) in the lichen *Parmelia cetrarioides.* There is also present a sterol giving a violet-red Liebermann reaction, changing to blue and then green. For the sterol we suggest the alternative formulae $C_{32}H_{50}O_4$ or $C_{33}H_{52}O_4$; the possibility that a mixture of sterols may be present is not excluded. The sugar-alcohol in the lichen is d-arabitol.

Experimental.

Ether Extract of Lichen.

700 g. of air-dried lichen were extracted with three successive lots of 5 l. of ether. The ether solution on evaporation gave 12·5 g. of a dark brown paste. This paste was stirred with 80 c.c. ether; on filtration a greenish brown

solution A and a residue B, which was a mixture of grey and yellow material, were obtained.

The solution A was extracted with successive portions of 50 c.c., 20 c.c., and 20 c.c., of 3 per cent. sodium carbonate solution. The alkaline extract foamed strongly on agitation and quickly set to a gel. It was acidified with dilute sulphuric acid, and extracted several times with ether; from this ether extract a brown gummy mass containing some crystals was obtained. This was boiled with 60/80 petrol, the solvent decanted from a gummy residue, and allowed to cool. The first material precipitated from the petrol was a gum, which was removed, and the mother liquors on standing deposited a crystalline product, which was purified by fractional crystallisation from 60/80 petrol. The purification was tedious, and eventually a crystalline product was obtained which had m.p. 104–106° C.

Analysis.

3·660 mg. gave 9·040 mg. CO_2; 2·350 mg. H_2O.
3·519 mg. gave 1·810 mg. AgI.
0·14 mg.—1·16 mg. camphor—21·0Δ.
Found C = 67·3; H = 7·1; OCH_3 = 6·8; M.W. = 230.
$C_{24}H_{29}O_6 . OCH_3$ requires C = 67·5; H = 7·2; OCH_3 = 6·98; M.W. = 444.

The substance is soluble in benzol, petrol, acetone, and alcohol. The alcohol solution is acid to litmus, and gives a red-brown colour with ferric chloride; reaction with bleaching powder is negative. The half value for the molecular weight indicates a depside. The formula, reactions, and melting point indicate that the material is perlatolic acid. This was confirmed by the products of hydrolysis, as obtained in the following experiment:—0·18 g. of the substance was heated at 60° C. with 4 c.c. of 5 per cent. methyl alcoholic potash for 3 hours in a hydrogen atmosphere. From the reddish solution obtained the greater part of the alcohol was removed, the solution diluted with 25 c.c. water, and the whole saturated with carbon dioxide. On extraction with ether a product was obtained which was hydrolysed by boiling with 4 c.c. aqueous potash; acidification and extraction with ether gave a material which on crystallisation from 60/80 petrol had m.p. 123–125° C. It was the p-methyl ether of olivetol carboxylic acid.

Analysis.

3·201 mg. gave 7·710 mg. CO_2; 2·150 mg. H_2O.
4·118 mg. gave 4·170 mg. AgI.
Found C = 65·6; H = 7·4; OCH_3 = 13·4.
$C_{12}H_{15}O_3 . OCH_3$ requires C = 65·5; H = 7·5; OCH_3 = 13·0.

The reddish solution referred to above was, after extraction with ether, acidified with dilute sulphuric acid, and extracted with ether. The product

recovered from the ether was crystallised from dilute methyl alcohol, and gave white needles m.p. 138–140° C., which were olivetol carboxylic acid.

Analysis.

3·490 mg. dried at 100° C. lost 0·150 mg.
3·340 mg. dried product gave 7·800 mg. CO_2; 2·100 mg. H_2O.
Found C = 63·7; H = 7·0.
$C_{12}H_{16}O_4$ requires C = 64·3; H = 7·1.
$C_{12}H_{16}O_4 . \frac{1}{2}H_2O$ requires H_2O = 4·0; Found 4·3.

The residue B referred to above, obtained by treatment of the lichen extract with a small amount of ether, was warmed with 30 c.c. chloroform; 0·7 g. of a cream-coloured powder C remained undissolved. The chloroform solution, on addition of alcohol, gave a precipitate of yellow needles, which, on recrystallisation from a mixture of alcohol and chloroform, had m.p. 205° C., and in chloroform solution was lævo-rotatory with a specific rotation of 485. It was l-usnic acid.

Analysis.

3·732 mg. gave 8·580 mg. CO_2; 1·520 mg. H_2O.
Found C = 62·9; H = 4·6.
$C_{18}H_{16}O_7$ requires C = 62·8; H = 4·6.

The powder C was treated with cold ethyl alcohol, in which it was partly soluble. The alcohol solution was evaporated to dryness, treated with methyl alcohol, which left behind a little usnic acid, and the methyl alcohol solution precipitated with water. The precipitate which still contained traces of usnic acid was warmed with chloroform to remove the latter, and was then crystallised from a small amount of ethyl alcohol, from which it separated in long white needles m.p. 245–250° C. The product, which is a sterol, is, in view of the unsatisfactory melting point, possibly a mixture of sterols. Though the preparation was repeated several times from different samples of lichen in no instance was a sharp melting point obtained. There was not enough material available to carry out a satisfactory chromatogammic separation.

Analysis.

(1) 3·340 mg. gave 9·450 mg. CO_2; 3·090 mg. H_2O.
(2) 3·720 mg. gave 10·610 mg. CO_2; 3·500 mg. H_2O.
(3) 3·698 mg. gave 10·500 mg. CO_2; 3·390 mg. H_2O.
(4) 0·664 mg.—7·040 mg. camphor—7·0°Δ M.W. = 498.
Found (1) C = 77·1; H = 10·27.
(2) C = 77·8; H = 10·4.
(3) C = 77·4; H = 10·2.
$C_{32}H_{50}O_4$ requires C = 77·1; H = 10·0 M.W. = 498.
$C_{33}H_{52}O_4$ requires C = 77·3; H = 10·1 M.W. = 512.

The substance gives with acetic anhydride and sulphuric acid a reddish violet colour which changes to blue and then green on standing. The analyses exclude the possibility of it being Zeorin.

The portion of the powder C insoluble in cold ethyl alcohol was boiled with alcohol until it gave no Liebermann reaction. It was then crystallised from aqueous acetone, from which it separated in small plates, m.p. 220° C. (with gas evolution). The substance is soluble in dilute sodium bicarbonate, gives a reddish brown colour with ferric chloride, but no reaction with bleaching powder. It contains methoxyl, but does not give the characteristic reaction of squamatic acid with sulphuric acid.

Acetone Extract of Lichen.

After extraction with ether the lichen was extracted with 5 l. of boiling acetone. The acetone solution on evaporation gave a somewhat gummy solid. This was extracted with water, and the aqueous solution evaporated to dryness; the material thus recovered was dissolved in ethyl alcohol, cleared with active charcoal, filtered, and the filtrate, after evaporation to a small bulk, treated with 10 volumes of dry ether. The material thus obtained was acetylated with acetic anhydride and sodium acetate, and the product obtained on pouring into water crystallised from ligroin, from which it separated in small plates, m.p. 72–74° C. A mixed melting point with d-arabitol pentacetate showed no depression.

No. 3.

ATMOSPHERIC POLLUTION IN DUBLIN DURING THE YEAR 1941.

By A. G. G. LEONARD, BRIDGET P. McVERRY, and D. CROWLEY.

[Read March 24. Published separately September 2, 1942.]

Work has been continued during 1941 at Leinster Lawn and the Albert Farm, Glasnevin.

Results obtained with Standard Gauges.

Details of the deposits during the year at Leinster Lawn and the Albert Farm are given in Tables 1 and 2 respectively.

Soluble solids deposited during the year at Leinster Lawn amounted to approximately 74 tons per square mile, and were about 1·6 times the amount at Glasnevin.

Insoluble solids at Leinster Lawn were deposited at the rate of 126 tons per square mile, and were 3·4 times the amount at Glasnevin.

The figure for insoluble matter at Glasnevin is 37 per cent. higher than that recorded in 1940, otherwise the deposits are not very different from those recorded in 1940.

Suspended Impurity.

The shortage of coal during 1941 has had a very marked effect on this impurity. A comparison of the figures for suspended impurity during 1941 with the average for the three years 1938, 1939, and 1940 is given in fig. 1a. From February, 1941, onwards there is a marked diminution in the degree of pollution as compared with previous years; actually the total pollution due to suspended impurity in 1941 was only one-half of the average for the preceding three years.

The number of "hazy days" (i.e. days upon which a shade number of 4 or more was recorded at any hour by the automatic filter) at Merrion Street in 1941 was 20, while the average number for the previous three years was 37.

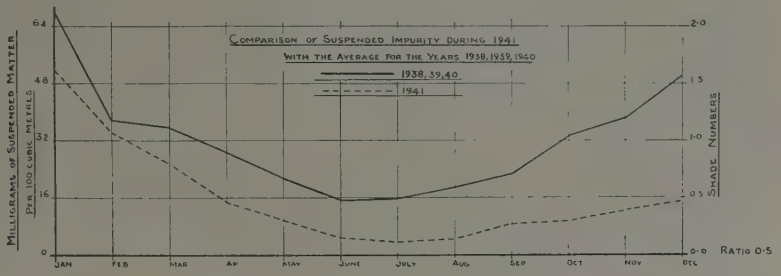
The following table gives the detailed results from February, 1938, when measurements were begun :—

Number of Hazy Days.

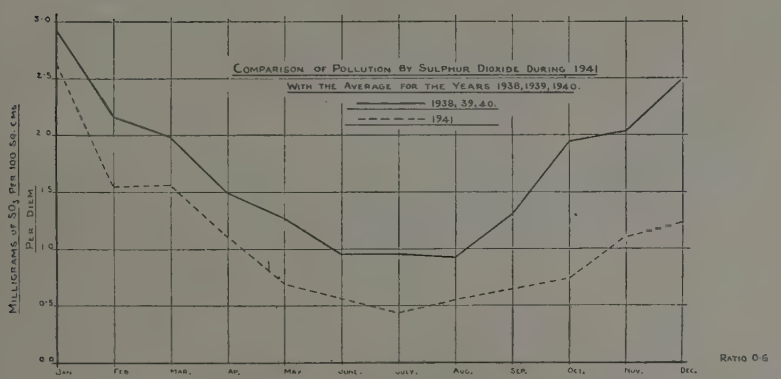
	Jan.	Feb.	Mar.	Oct.	Nov.	Dec.	Total.
1938...	—	4	7	3	7	9	30
1939...	9	3	1	7	3	9	32
1940...	17	8	7	3	9	6	50
1941...	11	4	3	0	1	1	20

The effect of the coal shortage is most marked in the last three months of 1941.

The same effect is to be noticed by a glance at the graphs for suspended impurity in fig. 1a.



a



b

FIG. 1.

Suspended Impurity and Deaths from Respiratory Diseases.

The curves of moving averages for both of these are given in fig. 2. The maximum on the impurity curve in the past winter occurs at January 24th, 1942, and shows a depth of pollution of about the same order as the minimum prevailing during the summer months of 1939 and 1940. Since minima of pollution in the summer months correspond approximately to minima in the deaths from respiratory diseases, it may be taken that the low maximum of pollution in the past winter is of no significance as affecting the rise of deaths from respiratory diseases. It is to be noted, however, that the maximum of deaths from respiratory diseases during the past winter is the lowest recorded in the years during which this investigation has been carried out, a fact which is probably due to the decline in the contributory factor of suspended impurity.

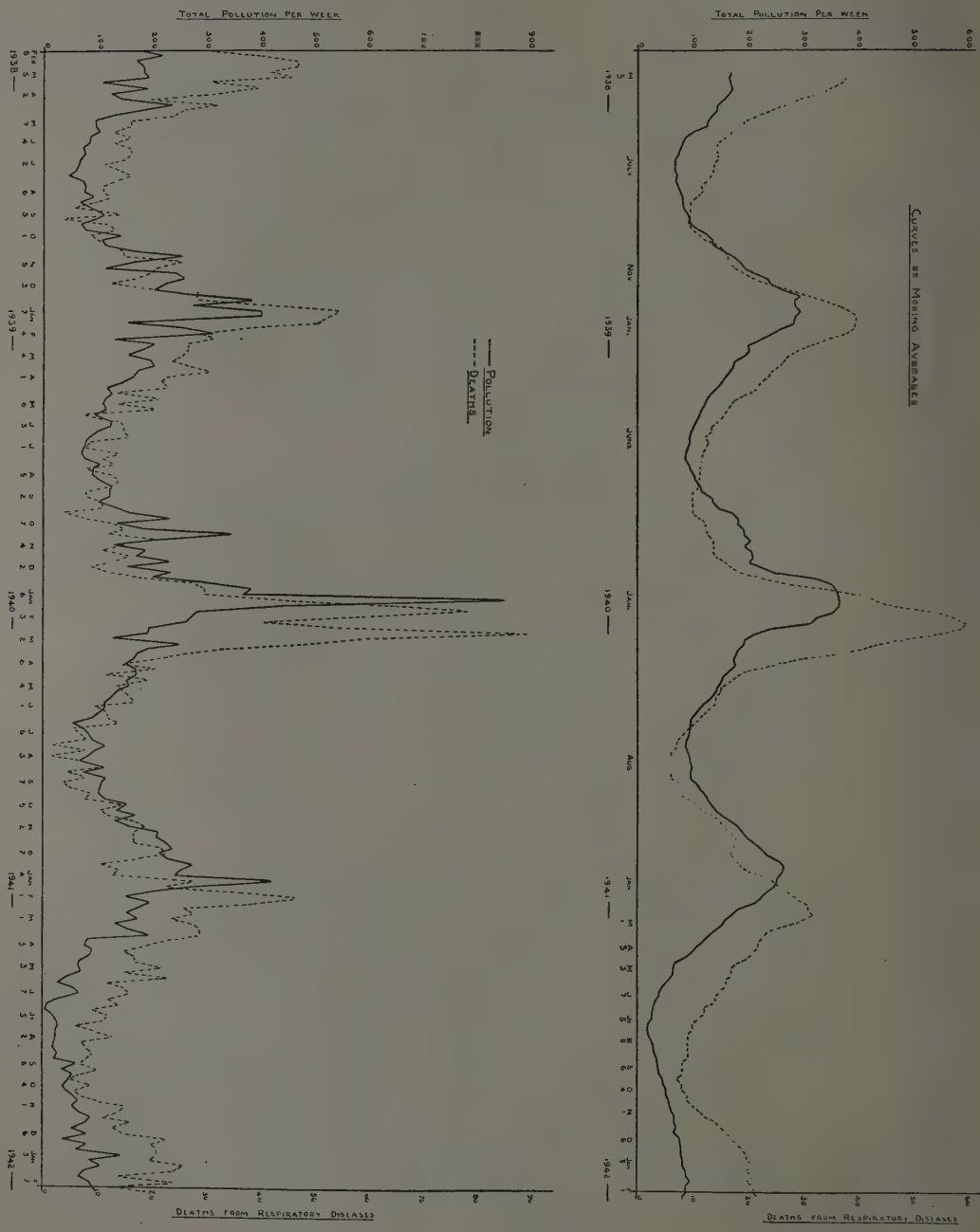

TOTAL POLLUTION PER WEEK
POLLUTION
DEATHS
DEATHS FROM RESPIRATORY DISEASES
1938
1939
1940
1941
1942
TOTAL POLLUTION PER WEEK
CURVES OF MOVING AVERAGES
DEATHS FROM RESPIRATORY DISEASES
1938
1939
1940
1941
1942

FIG. 2.

Sulphur Dioxide.

Fig. 1b shows the monthly graph for sulphur dioxide at Leinster Lawn in 1941 determined by the lead peroxide method, compared with the average values for the three years 1938, '39, and '40. It may be seen that the curves are very similar in trend to those for suspended impurity over the same periods. The total pollution by sulphur dioxide in 1941 was 0·6 of the average of the previous three years. Fig. 3 shows the graphs for sulphur dioxide at the Albert

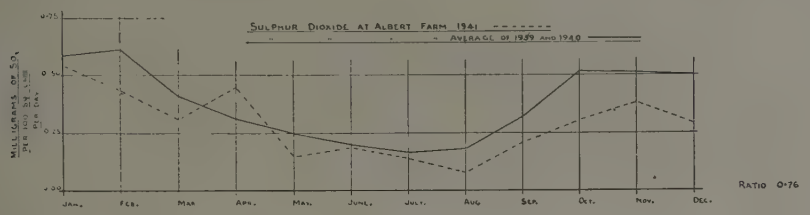


FIG. 3.

Farm during 1941, and the average for the years 1939 and 1940. Except for the month of April, the figures for 1941 lie below those for 1939–1940, the total for 1941 being 0·76 of the average of the previous two years.

Wind Velocity, Direction and Pollution by Suspended Impurity.

In our first paper on atmospheric pollution (Sci. Proc. R.D.S., 1939) some attempt was made to classify the above quantities. As the coal shortage has produced such a marked reduction in the intensity of suspended impurity during 1941 it has been decided to classify the observations obtained during the winter months of 1938, 1939, and 1940, which may be taken as representing years of normal coal consumption. We are indebted to Dr. H. H. Poole for suggesting the method of representation given in fig. 4.

The concentric circles represent depth of pollution increasing from the centre (0) to the outer circle (5). The dotted lines connect points representing the average values of the shade numbers recorded on the automatic filter at 9 a.m. for the wind directions and velocities (Beaufort, Nos. I to IV), indicated, as recorded by Mr. J. Gilmour at 9 a.m. at Trinity College. For calm (Beaufort, No. 0), the average shade was 5·0, as indicated by the darkening of the outer circle.

It is to be noticed that the winds N.N.W., N.W., and W.N.W. are on the whole most heavily polluted. The automatic filter is situated in the north wing of the Science Buildings, Upper Merrion Street, and such winds have passed over a large area of the city before measurement. So far as we are aware there

TABLE 1.—*Impurity Deposited from the Atmosphere at Leinster Lawn during the Year 1941.*

Grams per square Decametre (Metric Tons per Hundred Square Kilometres).

	Rainfall. mm.	PH of rain-water.	*Insoluble Matter.*			*Soluble Matter.*		Total solids.	*Included in Soluble Matter.*			
			Tar.	Carbon-aceous other than tar.	Ash.	Loss on ignition.	Ash.		Sulphates (SO_3).	Chlorine (Cl.).	Ammonia (NH_3).	Lime (CaO).
January . .	105	4·8	17	137	211	178	421	964	92	214	5	30
February . .	66	5·8	19	119	271	128	213	750	72	102	4	41
March . .	96	5·4	20	184	327	159	238	928	88	120	8	31
April . . .	69	4·8	15	130	179	53	125	502	51	33	1	69
May . . .	68	5·5	21	233	356	23	149	782	47	24	2	35
June . . .	20	6·8	8	169	189	16	97	479	33	8	1	20
July . . .	36	6·8	7	196	277	11	120	611	32	7	1	27
August . .	61	6·6	6	230	205	27	172	640	37	21	4	36
September .	17	5·5	5	188	173	7	96	469	37	6	1	18
October . .	52	5·3	5	244	235	86	141	711	47	21	6	33
November .	81	5·8	5	164	184	125	213	691	79	93	7	31
December . .	34	5·5	6	212	85	67	115	485	52	32	4	32
Mean Monthly	59		11 B	184 B	224 B	73 A	175 B	667 B	56 A	57	4	34
Summer Total	271		62	1146	1379	137	759	3483	237	99	10	205
Winter Total	434		72	1060	1313	743	1341	4529	430	582	34	198
Annual Total	705		134	2206	2692	880	2100	8012	667	681	44	403

TABLE 2.—*Impurity Deposited from the Atmosphere at Albert College, Glasnevin, during the Year 1941.*

Grams per Square Decametre (Metric Tons per Hundred Square Kilometres).

	Rainfall. mm.	PH of rain-water.	*Insoluble Matter.*			*Soluble Matter.*		Total solids.	*Included in Soluble Matter.*		
			Tar.	Carbon-aceous other than tar.	Ash.	Loss on ignition.	Ash.		Sulphates (SO_3).	Chlorine (Cl.).	Ammonia (NH_3).
January . .	64	4·95	4	21	20	131	146	322	63	94	1
February . .	44	4·92	3	23	40	126	72	264	35	65	3
March . . .	72	4·74	1	35	68	81	178	363	36	98	2
April . . .	66	5·28	2	32	52	67	84	237	38	43	7
May . . .	66	5·22	–	52	113	78	50	293	27	14	8
June . . .	22	6·78	–	123	67	96	69	355	21	11	22
July . . .	45	6·36	–	99	77	33	42	251	21	7	—
August . .	69	6·23	1	58	43	52	38	192	18	14	1
September .	22	5·94	2	102	141	34	39	318	29	8	2
October . .	46	5·79	3	61	49	40	50	203	25	17	2
November .	52	5·31	6	51	69	118	81	325	37	65	3
December .	36	5·11	1	30	24	40	40	135	18	21	4
Mean Monthly	50		2	57	64	75	74	272	31	38	5
Summer Total	290		5	466	493	360	322	1646	154	97	40
Winter Total	314		18	221	270	536	567	1612	214	360	15
Annual Total	604		23	687	763	896	889	3258	368	457	55

is, in this direction, no source of heavy pollution in proximity to the automatic filter, and the graphs, therefore, appear to give a fair representation of pollution in so far as wind direction is concerned.

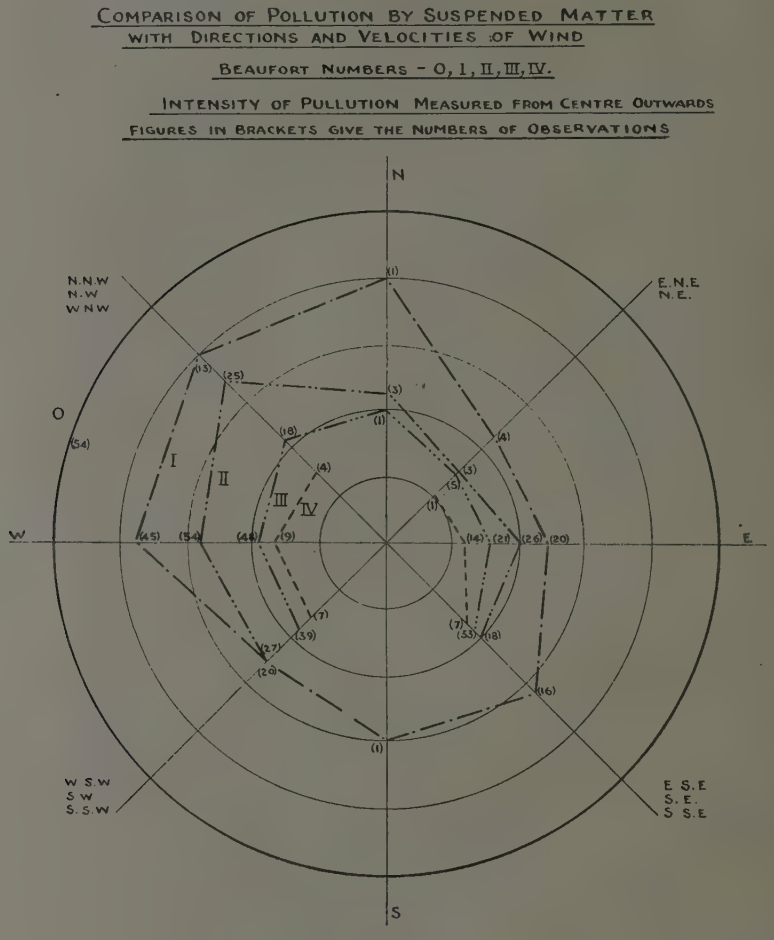


FIG. 4.

Measurements of Sunlight.

Fig. 5 gives the records from January, 1941, to March, 1942. The loss of light at Merrion Street was considerable during the early part of 1941, falling to a much lower figure during the period May to June. The losses during the

last two periods on the graphs are the lowest so far recorded for those periods during the three years for which records have been made.

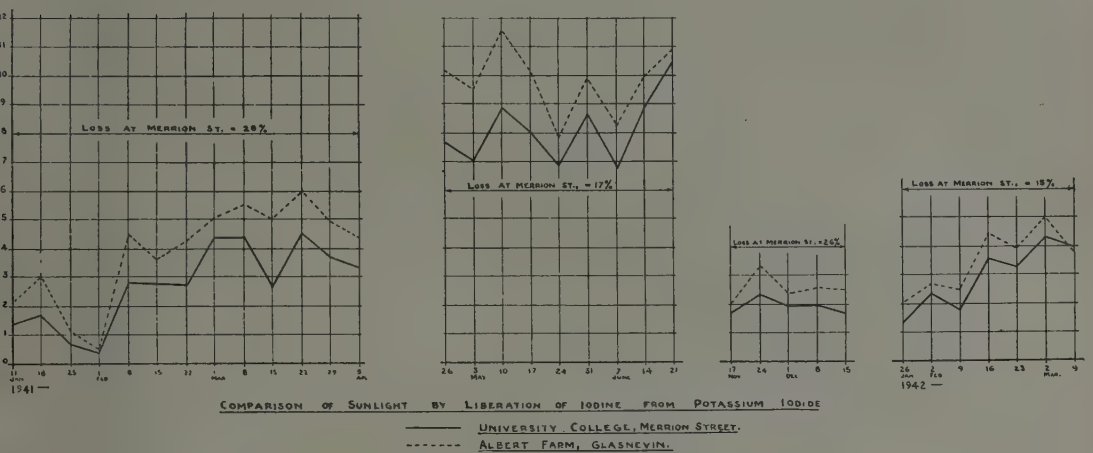

LOSS AT MERRION ST. = 28%
LOSS AT MERRION ST. = 17%
LOSS AT MERRION ST. = 26%
LOSS AT MERRION ST. = 15%
1941 —
1942 —
COMPARISON OF SUNLIGHT BY LIBERATION OF IODINE FROM POTASSIUM IODIDE
UNIVERSITY COLLEGE, MERRION STREET.
ALBERT FARM, GLASNEVIN.

FIG. 5.

Legislation to Prevent Smoke Pollution.

Persons interested in the prevention of smoke pollution will find in the library of the Royal Dublin Society a copy of an ordinance which is now in force in the city of St. Louis, and which the corporation state has already solved for the most part the nuisance previously due to smoke in that city.

CHEMICAL DEPARTMENT,
UNIVERSITY COLLEGE,
DUBLIN.

No. 4.

THE IDENTITY OF THE TOP-NECROSIS VIRUS IN UP-TO-DATE POTATO.

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(Plate I.)

[Read May 19. Published separately October 6, 1942.]

Top-necrosis was the term applied by Quanjer and Oortwijn Botjes (18) in 1929 to a specific type of potato virus disease characterised by necrosis of the inner phloem strands, death of the growing points, and eventual killing of the plant from the top downwards. The general term "streak" had previously been used in reference to all symptoms of a necrotic nature, irrespective of the location of the necrosis in the plant. It was recognised that more than one virus was capable of producing top-necrosis, and also that the range of varieties reacting in this manner differed for each of the viruses in question.

The presence of a top-necrosis ("streak") virus in apparently healthy Up-to-Date potatoes was detected in the course of intervarietal grafting experiments by Murphy and McKay (14) in 1926, a similar observation in regard to the American variety, Green Mountain, having been made by Schultz and Folsom (21) in the previous year. It is now known that practically all plants of these varieties are carriers of top-necrosis, and that the responsible virus is probably the same in both cases (16, 11). Other varieties are similarly infected, at least to some extent, e.g. Bliss Triumph, Burbank, Duke of York, and Earliest of All. So far as the writer is aware, no plant which is a carrier of this particular virus has been found free from virus X, as shown by the characteristic mosaic symptoms produced on inoculation to such hosts as *Datura Stramonium* and tobacco.

In 1931 Smith (24) recorded that analysis of the virus content of symptomless "streak"-carrying Up-to-Date yielded virus X and the insect-transmissible virus Y. Salaman and Bawden (20) reaffirmed this statement, but considered that the top-necrosis complex possibly contained a third virus (Z) in addition. However, the view that Up-to-Date "streak" is a complex of X and Y has not been confirmed by other investigators, nor has it been maintained at Cambridge. In 1933 Clinch and Loughnane (6) showed that the top-necrosis virus from Up-to-Date was filterable through an L5 Pasteur-Chamberland filter candle, and transmissible by sap inoculation to *Datura Stramonium* and tobacco. As no separation of viruses occurred in the course of these experiments, they suggested that the Up-to-Date contained only one virus, which was responsible for the top-necrosis in potato as well as for the mosaic symptoms in *Datura* and tobacco. Bawden (2) disagreed with this view

on the grounds that he could isolate from Up-to-Date a typical X virus which was non-necrotic to President and Arran Victory potato; he considered, therefore, that a second virus, probably distinct from X, must be responsible for the top-necrosis symptoms produced in potato. Bawden had no means of isolating the second virus, but designated it virus B (= *Solanum virus* 4 in Smith's (25) classification).

Dykstra (11, 12) stated that the top-necrosis virus could be separated from X in the Up-to-Date complex by grafting Up-to-Date on the U.S.D.A. Seedling 41956, which is immune to X but not to B. He recorded that virus B alone produced top-necrosis in President and Arran Victory, but caused no symptoms in tomato, *Datura Stramonium*, or pepper. He did not investigate any other properties of the virus. A fuller study of the properties of virus B (*Solanum virus* 4) was undertaken by Dennis (10), who also used Seedling 41956 for the isolation of the virus from Up-to-Date. Contrary to Dykstra's experience, Dennis found that the purified virus B which he recovered from Seedling 41956 produced conspicuous mosaic symptoms of the X type on many solanaceous hosts including tomato, *Datura Stramonium*, and pepper. The virus also reacted with anti-X rabbit serum, and its physical properties were identical with those of virus X. On the latter point, Dennis's results were somewhat at variance with those of Bawden (2), who had found that viruses B and X had similar thermal death points but differed in their resistance to "ageing" and dilution. According to Dennis the two viruses, B and X, differed only in their effects on certain potato varieties, and in the failure of either to protect against infection with the other. However, the ability of virus B to infect X-immune Seedling 41956, if established, would obviously constitute a still more important point of distinction between the two.

The present writer has had occasion to examine the top-necrosis virus from Up-to-Date in order to compare it with another top-necrosis virus related to F (8). In the course of this examination certain facts have come to light which appear to be of importance in establishing the identity of virus B, and the results are accordingly presented herewith.

SOURCE OF VIRUS AND SYMPTOMS IN OTHER HOSTS.

The stock of Up-to-Date potato used in this work was the same as that used by Clinch and Loughnane (6) in 1933, and has been grown continuously in an insect-proof glasshouse since that time. The plants are vigorous, and almost perfect virus carriers, displaying at times, however, a slight general mottling, especially when approaching maturity.

The reactions of other potato varieties when grafted with scions of Up-to-Date are as follows:—

(a) *Top-necrosis.*—Arran Cairn, Arran Chief, Arran Comrade, Arran Crest, Arran Pilot, Arran Scout, Arran Victory, Ballydoon, British Queen, Catriona, Doon Early, Dunbar Standard, Dunbar Yeoman, Early Regent,

Epicure, Gladstone, Golden Wonder, Katahdin, Kerr's Pink, King Edward, May Queen, President, Sharpe's Express, Ulster Monarch. (This group includes four varieties which react with top-necrosis to virus X, viz.: Arran Crest, Early Regent, Epicure, and King Edward.) (b) *Symptoms as in Up-to-Date.*—Arran Banner, Arran Consul, Arran Peak, Arran Signet, Bliss Triumph, Champion, Di Vernon, Duke of York, Dunbar Cavalier, Eclipse, Great Scot, Green Mountain, Majestic, Redskin. (c) *Crinkle.*—Irish Chieftain. (This variety is infected with virus A.)

Many of the foregoing varieties were tested at Cambridge as well as at Glasnevin, with similar results at both centres. Bawden (2) also records that the American varieties Burbank, Earliest of All, and American Wonder are carriers of the Up-to-Date virus.

Sap inoculation from Up-to-Date to other potato varieties.—It is generally stated that the Up-to-Date "streak" virus (= virus B) is not sap inoculable to potato, and is therefore less infectious than virus X. This, however, is only true of those varieties such as Arran Victory, British Queen, and President, which react to the "streak" virus with top-necrosis. Inoculation of these intolerant varieties is followed by the development of local necrotic lesions (Pl. I, Fig. 1), but the necrosis only rarely becomes systemic. The number of local lesions produced is greatly increased by sprinkling carborundum powder on the leaves before making the inoculation. Healthy plants of carrier varieties, i.e. those which react like Up-to-Date itself, can be infected with virus B just as easily as with virus X, and 100 per cent. infection of such varieties has been obtained in several different experiments.

On recording that inoculation of Arran Crest with Up-to-Date sap results in top-necrosis (which is also the effect of virus X in this variety), Clinch and Loughnane (6) suggested that the necrotic reaction could be due to the "streak" virus, i.e. virus B. Bawden (2), however, considered that the necrosis was due entirely to an X virus accompanying virus B in Up-to-Date, and stated that only virus X was recoverable from the necrotic foliage of the Arran Crest. That virus B may be introduced to Arran Crest by sap inoculation was shown in the following manner: Three healthy plants of the variety Arran Victory (necrotic to B, non-necrotic to X) were grafted with scions of healthy Arran Crest which, when fully established, were inoculated with Up-to-Date sap. Numerous local necrotic lesions developed all over the rubbed leaves. Later, the tops of the Arran Crest scions were cut, following which systemic necrosis developed in two of them. All three Arran Victory stocks developed "streak" symptoms, and *Datura* plants inoculated from them showed mosaic. Clearly, the "streak" virus entered the Arran Crest following inoculation, and passed eventually into the Arran Victory indicator stocks, although the experiment does not prove whether it or an accompanying X (or both together) is responsible for the top-necrosis in the Arran Crest.

Sap inoculation from Up-to-Date to other hosts.—The symptoms produced in *Datura Stramonium* and tobacco (*var.* white Burley) have been already

described by Clinch and Loughnane (6). They consist of a mosaic mottling identical with that caused by a mild strain of X (Pl. I, Fig. 2). The symptoms in tomato are also of the X type, and are distinctly mild; initial vein-clearing in the top leaves is followed by a slight dark and light green mottling, which later fades but is again visible on the lower leaves at maturity.

All plants of the above hosts which showed mosaic following inoculation were found to contain the top-necrosis virus when tested by grafting on suitable varieties of potato, such as President and Arran Victory.

Infection of a non-solanaceous host with virus B.—Having found that certain species of the common hemp-nettle, *viz. Lamium purpureum* and *L. hybridum* (Natural Order *Labiatae*), were susceptible to infection with virus X, tests were made to determine whether these species were also susceptible to virus B. Sap from "streak"-carrying Up-to-Date was inoculated to *Lamium hybridum* by the rubbing method, using carborundum powder. About three weeks later a mild mottle appeared in the leaves of the *Lamium* plants, and sub-inoculations from the mottled leaves to *Datura Stramonium* resulted in mosaic symptoms similar to those induced by direct inoculation from Up-to-Date. Scions from the *Datura* plants in turn produced top-necrosis when grafted on Arran Victory potato, showing that the virus content of the Up-to-Date was unchanged by passage through the non-solanaceous host. It has not so far been found that *Lamium* is susceptible to potato viruses other than X and B, but work in this direction is still in progress.

ATTEMPT TO ISOLATE THE TOP-NECROSIS VIRUS FROM UP-TO-DATE BY INFECTION OF U.S.D.A. SEEDLING 41956.

The stock of Seedling 41956 used in the tests was made available to this laboratory through the courtesy of Dr. T. P. Dykstra of Corvallis, Oregon. The variety was found to be highly susceptible to potato viruses Y, A, stipple streak, F, and a top-necrosis strain of F (8). Repeated attempts to infect it with either mild or severe strains of X were, however, entirely unsuccessful, a result which confirmed the statements of Schultz *et al.* (22), Dykstra (12), and Dennis (10) regarding the immunity of Seedling 41956 to virus X.

With a view to removing X from the supposed X + B complex in Up-to-Date, five plants of Seedling 41956 were grafted with Up-to-Date scions. After 34 days, when the scions had grown vigorously, each of the stock plants was tested for virus by grafting on President and Arran Victory potato, and by inoculation to *Datura Stramonium,* but with negative results. The tests were repeated after 84 days, but were again negative. Scions from the Seedling 41956 stocks were also grafted on President plants infected with mild X, on the assumption that B, if isolated in 41956, might require the addition of X in order to produce the top-necrosis reaction characteristic of the original Up-to-Date in President. In no case was a virus detected in the Seedling 41956 stocks, nor was any virus present in the tubers of these plants when they were

tested in the following year. The continued presence of the "streak" virus in the Up-to-Date scions after 84 days was, however, confirmed when the tops of the latter were grafted on healthy President, which reacted with top-necrosis. This proved that union with the tissues of Seedling 41956 had not affected the virus in the Up-to-Date scions.

In the following year three Seedling 41956 tubers were core-grafted from Up-to-Date, but the plants grown from them were virus-free. Four more 41956 plants were top-grafted as before, and 58 days later, when the Up-to-Date scions were 12–14 ins. high, shoots from each stock were grafted on President and Arran Victory. No symptoms developed in the latter, and when the tests were repeated after a further 18 days they were again negative. Four vigorous young 41956 plants were also inoculated with Up-to-Date sap, using carborundum powder. Four weeks later scions from each were grafted on healthy British Queen plants, but the latter failed to show any symptoms. All attempts therefore to infect Seedling 41956 with a virus from Up-to-Date were unsuccessful, although any other variety of potato could be infected with certainty by top-grafting.

It was understood from Dykstra's (11) preliminary statement on the subject that virus B was introduced without difficulty into Seedling 41956 by top-grafting with Up-to-Date, and was isolated in pure form, i.e. free from X, on Arran Victory potato and tomato by sub-grafts from the infected 41956. In a later paper (12) separation was said to have been effected by double-grafting Seedling 41956 and Up-to-Date on Arran Victory stocks, and this technique was accordingly tried by the writer. In the first experiment two healthy Arran Victory plants were grafted with Seedling 41956 scions which, when established, were further grafted with Up-to-Date scions. Both Arran Victory plants developed top-necrosis, which appeared after 10 and 18 days respectively. The intermediate 41956 scions were 2–3 ins. long, and each had one foliage leaf. In a second experiment one Arran Victory and one British Queen were used as basal stocks, and the 41956 scions were allowed to become tall and extremely vigorous before being top-grafted with the Up-to-Date. The cutting of the tops at the time of grafting with Up-to-Date stimulated growth of the axillary buds on the 41956 stems, and these rapidly developed into strong leafy shoots. Eighteen days after grafting with Up-to-Date streak lesions appeared on the Arran Victory and British Queen stocks, and top-necrosis later ensued (Pl. I, Fig. 3). In further experiments the variety Arran Crest, which is intolerant of X, was used as the basal stock. Five Arran Crest plants were double-grafted, and the intermediate Seedling 41956 scions were in all cases 6–8 ins. long, with vigorous leafy shoots. Four of the Arran Crest plants developed top-necrosis, the streak lesions appearing in one case 13 days after grafting with Up-to-Date. The fifth Arran Crest failed to show symptoms, but in this case the Up-to-Date scion formed stolons at the base, and the axillary buds swelled up to form small aerial tubers, showing that downward translocation from the scion was inhibited.

The appearance of the top-necrosis in the basal stocks of Arran Victory

and British Queen confirmed Dykstra's (12) statement that virus B could pass through an intermediate scion of Seedling 41956 in a double graft. It was found, however, that when sap from any of the basal stocks used (including Arran Crest) was inoculated to *Datura Stramonium*, mosaic symptoms developed which were identical with those produced by direct inoculation from Up-to-Date. The rate of infection from any one potato plant was comparatively low, and badly necrosed tissues which had dried out usually failed to produce symptoms. Altogether eleven out of thirty *Datura* plants inoculated from necrotic foliage showed mosaic, and scions from these plants produced top-necrosis when grafted on Arran Victory or British Queen. The symptomless *Datura* plants, on the other hand, were found to be virus-free. It appeared, therefore, that the Up-to-Date virus complex had not been altered by passage through the intermediate scions of Seedling 41956. Tests were then made of the virus content of the 41956 intermediate scions. Sap inoculation to *Datura* failed to produce symptoms, and subsequently scions from the 41956 branches were grafted on six Arran Victory plants. Although taken from double-grafted plants with flourishing Up-to-Date scions and severely necrotic basal stocks, these scions were all virus-free.

Further double-grafting experiments were carried out in which the Up-to-Date top scions were replaced by President scions infected with a severe strain of X, while Arran Victory and Arran Crest were used as the basal stocks. Notwithstanding the fact that in certain cases the intermediate 41956 scions were 12–14 ins. long, with vigorous leafy shoots, virus X in all cases passed from the top scions, and produced its characteristic symptoms in the basal stocks, but the 41956 did not itself become infected. These results show that Seedling 41956, inserted as an intermediate scion in a double graft, does not prevent the passage of virus X. The fact that the top-necrosis virus from Up-to-Date passed through Seedling 41956 in double grafts is therefore not proof that this virus is distinct from virus X.

Effect of Anti-X Serum on the Infectivity of Up-to-Date Sap.

Recent work by Chester (5) and others has established the specificity of the reaction between plant viruses and their homologous antisera. It has also been shown that different strains of a given virus, however unlike symptomatically, are serologically indistinguishable at least with the ordinary precipitin technique. Spooner and Bawden (26) found no antigenic differences between different strains of potato virus X, and demonstrated incidentally that sap from Up-to-Date potato carrying a mild strain of X flocculated with anti-sera to the S and G strains of X derived from tobacco. No flocculation occurred when anti-X sera were mixed with saps containing viruses unrelated to X. It might be expected, therefore, that if Up-to-Date sap contains two distinct viruses, X and B, the X could be removed from the mixture by neutralization with its specific immune serum. This technique was successfully employed by Cohen (9)

to purify tobacco necrosis virus from admixture with tobacco mosaic virus. Cohen also found that the removal of the tobacco mosaic virus from the mixture by this method entailed no apparent loss in the amount of tobacco necrosis virus.

The anti-X serum used in the present work was obtained from rabbits which had received a series of 6 intravenous injections with a mild strain of virus X from President potato. Preliminary precipitin tests with healthy and X-infected sap of British Queen potato showed that the serum reacted well with the X sap, but gave no precipitate with the healthy juice. A series of tubes was prepared, each containing 2 c.c. of antiserum at dilutions (in 0·85% saline) of 1 in 2, 1 in 4, 1 in 8, and 1 in 16, respectively, control tubes receiving saline only. The tubes were well shaken, incubated at 37° C. for 1½ hours, and then placed in the refrigerator for 1 hour. Only those containing anti-X serum showed flocculation. The tubes were then centrifuged for 10 minutes, and the supernatant fluids inoculated to *Datura Stramonium*. The mixtures containing serum dilutions 1 in 2, 1 in 4, and 1 in 8 failed to produce symptoms in *Datura*, showing that the X virus had been completely neutralized. Scions from these symptomless *Datura* plants were then grafted on healthy Arran Victory potato plants, but these failed to show any reaction, indicating that the virus responsible for top-necrosis had also been removed.

The antigen solutions used in these tests were not highly purified like those of Cohen, and the possibility must be considered that even if virus B were a separate virus, serologically distinct from X, it might be carried down mechanically in the serum-virus X precipitate. It seems unlikely, however, that a resistant virus existing in fairly high concentration would be completely removed in this manner, and the experiment strongly suggests that the top-necrosis virus is actually neutralized by the anti-X serum.

Physical Properties of the Up-to-Date Top-Necrosis Virus.

On account of the failure to isolate virus B from the supposed X + B complex in Up-to-Date, tests of the physical properties of the virus were made with crude Up-to-Date sap. The juice was subjected to the desired treatments, and each treated sample (and untreated control) inoculated to 4–6 *Datura* plants. Scions from the latter were subsequently grafted on healthy Arran Victory plants, whereby the presence of virus B could be detected.

Filterability.—Virus B passed freely through L 1, L 3, and L 5 grades of Pasteur-Chamberland filter candles.

Thermal inactivation point.—68°–70° C. (10 mins. exposure).

Longevity in vitro.—Varied with different samples taken at different times. The crude sap was stored in stoppered bottles in the dark at 17°–20° C. In some cases infectivity was lost after 28 days, while in others infection was secured after 35 but not after 45 days. The approximate time for complete inactivation under the conditions mentioned is about 30 days.

Resistance to dilution.—50% infection was obtained at a dilution of 1 in 10,000, none at 1 in 100,000.

In general, these properties are essentially similar to those recorded for viruses of the X type. Furthermore, in the course of these tests virus B was recovered from all *Datura* plants which showed mosaic symptoms following inoculation with treated sap, while symptomless *Datura* plants were found to be virus-free when grafted on Arran Victory. Consequently, if two viruses are present in the Up-to-Date sap, one responsible for the top-necrosis and the other responsible for the mosaic symptoms in *Datura,* both must have identical physical properties. Actually the results fail to indicate that more than one virus is present, and are not in agreement with Bawden's statement (2) that virus B is destroyed and that virus X alone is recovered from Up-to-Date sap after 4 weeks' "ageing" or at a dilution of 1 in 1000.

IMMUNOLOGICAL RELATIONSHIP OF VIRUSES X AND B.

In order to test whether virus X exercised protection against virus B, sap from Up-to-Date was inoculated to X-infected potato plants of three different varieties. These included Majestic, which is a carrier of virus B, and President and British Queen, both of which react to B with necrotic symptoms. The plants used in these experiments were young, growing actively, and of uniform size. Except where otherwise mentioned carborundum powder was sprinkled on the leaves before rubbing them with the infective sap.

(1) Six healthy plants of Majestic and six infected with a mild strain of X were inoculated with Up-to-Date sap on 24th March. After about 14 days the healthy plants developed pale spots on the upper leaves, and later a diffuse mild mottle similar to that in the X-infected plants. The latter showed no change due to the inoculation. After 38 days a scion from each plant was grafted on Arran Victory; the six previously healthy plants caused top-necrosis, but the X-infected plants produced only a mild mosaic mottle. The X-infected plants, therefore, had resisted infection with the "streak" virus, while the healthy plants were fully susceptible.

(2) Six healthy President and six infected with a mild X strain were inoculated as above. The two lots were grown on either side of a glasshouse compartment, which was partially shaded so as to minimise hardening of the foliage. After about 14 days small local lesions appeared on the healthy plants, and these gradually increased in size to a diameter of 5–7 mm., and then dried out. In the meantime a second inoculation was made to the next three leaves above those already inoculated, and these in turn showed similar lesions. Not a single lesion appeared on any of the X-infected plants under the same conditions. Twenty-eight days after inoculation the upper leaves of the originally healthy plants were tested by inoculation to *Datura Stramonium.* Two of the six plants were found to contain a mild X virus which was non-necrotic to Arran Victory. Later the mottle was visible in the potato plants

themselves. This experiment was repeated exactly with twelve more President plants. Again, the six X-infected plants failed to show local lesions, while all the healthy plants did so. In addition one of the healthy plants later became systemically infected with a mild X virus.

(3) Three healthy British Queen plants and three infected with virus X were inoculated with Up-to-Date sap on 23rd May. The local necrotic reaction of British Queen to Up-to-Date sap is much more pronounced than that of President, possibly because the British Queen foliage is much more sappy. Five days after inoculation numerous minute necrotic lesions appeared on the three healthy plants, and these rapidly developed into distinct greyish lesions, which coalesced and eventually caused the leaves to turn yellow and die; not a single lesion appeared on the X-infected plants. Nineteen days after inoculation the upper leaves of the healthy plants were tested on *Datura*, but no symptoms were produced. After a further 10 days one of the plants showed systemic streak lesions in the lowest axillary shoots, then in the next shoots above, and so on until lesions were visible in the top leaves. These lesions tended to dry out, they were accompanied by pale blotches, and eventually the foliage presented a strongly mottled appearance with accompanying necrotic lesions. The growing points were not killed, and the advance of the disease was distinctly acropetal and associated with the development of axillary shoots. The other two plants also showed slight systemic streak symptoms, which did not appear for more than 6 weeks after inoculation and were confined to a few isolated necrotic lesions associated with the veins of the leaves.

In a further experiment six healthy British Queen plants and six infected with X were inoculated with sap from "streak"-carrying Up-to-Date. In addition six healthy British Queen plants were inoculated with a mixture of sap from "streak" carrying Up-to-Date and from Up-to-Date free from virus B but carrying a mild X. Four leaves on each of the eighteen plants were smeared with inoculum, but only two leaves in each case were sprinkled with carborundum powder. All plants were grown under the same conditions. Four days after inoculation numerous minute necrotic local lesions appeared on the leaves of the twelve healthy plants to which carborundum powder had been added (Fig. 1), and these rapidly increased in size, coalesced and dried out. Very few lesions developed on the leaves which had not received carborundum powder. As before, the leaves of the X-infected plants failed to show a single lesion. Twenty-one days after inoculation, an upper leaf from each of the twelve previously healthy plants was removed and the sap inoculated to two *Datura Stramonium* plants. The results showed that only one out of the six British Queen plants inoculated with "streak" Up-to-Date sap was systemically infected with mild X virus, while all six plants inoculated with the mixture of Up-to-Date saps were so infected. Later, following cutting back, top-necrosis developed in one of the plants inoculated with "streak" sap only, while mosaic developed in another.

The results of the foregoing experiments show that of twenty-one X-infected potato plants inoculated under optimum conditions with sap from Up-to-Date, not a single one showed a trace of infection with virus B, although healthy control plants inoculated at the same time all showed evidence of infection with the necrotic virus. The systemic development of a non-necrotic X virus in some of the control plants, which will be discussed later, does not alter the fact that virus B cannot be introduced by sap inoculation to potato plants already systemically infected with virus X; in other words virus X protects against virus B, and the two must be regarded as related strains. In a single immunity experiment in which *Datura Stramonium* was used the results confirmed those obtained with potato. Four out of a batch of eight *Datura* plants were inoculated with virus X, and the remaining four, which were to serve as controls, were inoculated with virus F, which is unrelated to X. Fifteen days later all the plants were inoculated with sap from streak-carrying Up-to-Date, and after a further 26 days tested by grafting on healthy Arran Victory potato. Only the four control *Datura* plants had become infected with virus B, and caused top-necrosis in Arran Victory.

The immunity conferred by virus X against virus B does not hold if the second virus be introduced by graft. While there is a distinct resistance to the entry of the second virus, it seems that this resistance is usually overcome when a constant supply of virus from a grafted scion is maintained. The top-necrosis, however, takes considerably longer to develop in X-infected plants than in healthy plants grafted under the same conditions, and sometimes the necrosis is restricted to the tips of the axillary shoots and fails to kill the growing points or to affect the foliage generally. Similarly, when scions infected with a severe strain of X were grafted on potato plants already infected with mild X, the severe strain had obvious difficulty in becoming established in the stock plants, but eventually did so and also penetrated the tubers. Bawden (1) previously reported that virus D, now known to be a strain of X, could be introduced by grafting but not by sap inoculation to potato plants already infected with virus X.

Bawden (2) and Dennis (10) both reported that virus X failed to confer immunity against the Up-to-Date streak virus. Part of the evidence for this statement was derived from experiments carried out with potato plants in which the second virus was introduced by graft. For reasons given in the preceding paragraph it is clear that the results of such experiments are unreliable. In the case of a single potato plant which was sap-inoculated with the second virus Dennis (10) found that previous infection with his *Solanum virus* 4 did not protect against further infection with virus D (1). In experiments with *Datura Stramonium*, Bawden (2) found that 11 out of 18 X-infected plants became infected with B when reinoculated with "streak" Up-to-Date sap, and concluded that the two viruses were not mutually protective. Dennis (10) supported Bawden's conclusion, stating that virus X in *Datura* did not exert any protective action against the *S. virus* 4 isolated by him from

Seedling 41956. Nevertheless, it would appear from Dennis's description that the full severe symptoms of his *S. virus* 4 were not produced when that virus was inoculated to *Datura* plants already infected with one or other strain of X, although a certain degree of alteration in the symptom picture resulted from the second inoculation. Furthermore, when tobacco plants infected with *S. virus* 4 were reinoculated with the intensely necrotic N strain of virus X (19), the full symptoms of the latter seem to have occurred in only one out of 10 plants inoculated. Unless *S. virus* 4 is unique among potato viruses in that it is less severe in combination than alone, these results seem to suggest that some measure of protection was afforded by the preliminary virus in the majority of these plants.

Dennis states that in his experiments the second virus was introduced 9 days after the first, a period which, in the writer's opinion, may have been insufficient to allow of complete invasion of the plants by the "protecting" virus. In two different tests carried out with young actively growing *Datura* plants it has in fact been found that, although vein-clearing was visible in the top leaves of all plants within 6–7 days after inoculation with a mild strain of X, complete invasion had not yet taken place in the majority of the plants after 9 days; the larger leaves, or parts thereof, still reacted with necrotic lesions to a severe strain of X. While it is possible that the initial virus might become completely systemic in young seedlings in less than 9 days, it appears safer to use potato plants grown from infected tubers for immunity experiments of this type; or else to extend the period between the first and second inoculations.

Isolation of a Non-Necrotic Virus X from "Streak"-Carrying Up-to-Date Potato.

It has been shown above that out of 21 healthy potato plants of the intolerant varieties President and British Queen which were inoculated with "streak"-carrying Up-to-Date sap and which showed local necrotic lesions, only 3 British Queen plants developed systemic streak symptoms. Five of the remaining 18 plants, however, eventually developed systemic infection with a mild, non-necrotic X virus. Bawden (2) had previously reported that President plants inoculated with Up-to-Date sap usually developed systemic infection with virus X, and also (as already mentioned) that a similar non-necrotic X could be isolated from Up-to-Date by certain physical treatments of the sap. He accordingly assumed that two distinct viruses are present in Up-to-Date, *viz.* virus X, which becomes systemic in President, and virus B, which remains localized in the lesions on the inoculated leaves.

In the present work the development of a non-necrotic X virus in the tops of potato plants inoculated with Up-to-Date sap was the exception rather than the rule. If such a virus co-exists with the top-necrosis virus in Up-to-Date, one would have expected a higher rate of infection, seeing that X is easily transmissible by sap inoculation to President and British Queen. The idea that virus X in the inoculum might in some cases be destroyed in the local lesions

caused by virus B was rejected in view of the high rate of infection obtained when sap containing mild X was mixed with the "streak" Up-to-Date sap before inoculation. Under the circumstances, and in view of the strong evidence that virus B is itself a form of X, the possibility must be considered that the non-necrotic form develops in the inoculated plants as a variant of the necrotic one. Price (17) found that new strains of cucumber mosaic could arise in local lesions on cowpea leaves, as well as in yellow spots on systemically diseased leaves. Salaman (19) also records that virus X was converted from a severe to a mild form in local lesions on certain non-solanaceous plants. It is true that such changes do not usually occur with any degree of regularity, and while the simpler explanation that two virus strains exist in Up-to-Date may be the correct one, nevertheless the possibility of strain variation in the local lesions cannot be excluded.

MODE OF TRANSMISSION OF THE TOP-NECROSIS VIRUS FROM UP-TO-DATE.

In a previous paper (6) it was reported that attempts to transmit a virus from Up-to-Date using *Myzus persicae* as vector were unsuccessful. The host plants used were President potato and *Datura Stramonium*. No further insect transmission experiments have been carried out, nor has there been any record of successful insect transmission by other workers, so far as the writer is aware.

In an examination of the spread of viruses into healthy potato stocks in the field, Clinch, Loughnane, and Murphy (7) found 4% infection with virus B in one crop of Champion and two crops of Arran Banner: the infected plants also gave mosaic symptoms typical of X in *Datura Stramonium*. Loughnane and Murphy (13) recorded accidental infection of a potato plant (*var.* Monocraat) with virus B. As the plant was grown in an insect-proof glasshouse, they presumed that infection took place as a result of contact with a B-infected plant. The present writer had occasion to grow Irish Chieftain potato plants alongside Up-to-Date plants in the glasshouse. In the following season the tubers of one Irish Chieftain gave rise to plants infected with crinkle (15), indicating the addition of X to the A already present in Irish Chieftain. When grafted on President, scions from the crinkle Irish Chieftain produced top-necrosis. There can be little doubt that this was another instance of transmission of B through leaf contact.

While definite experiments on the transmission of B by leaf contact have yet to be carried out, the evidence so far available suggests that B is probably transmitted under natural conditions in the same way as X, *viz.* by contact between leaves of adjacent healthy and diseased plants.

DISCUSSION AND CONCLUSIONS.

The results of the present investigation appear to furnish convincing evidence that the top-necrosis virus in Up-to-Date ("streak" virus, virus B,

Solanum virus 4) is a strain of virus X. It is similar to X in transmissibility, host range, physical properties, and in its inability to infect X-immune Seedling 41956; there is also some evidence that it is indistinguishable from X serologically. Furthermore, it cannot be introduced by sap inoculation to potato plants already infected with virus X. It differs from typical virus X only in that it causes top-necrosis in a number of potato varieties not so affected by the typical strain, a difference which recently has been shown to exist between two strains of potato virus F (8). It is possible that a non-necrotic strain of X may co-exist with the top-necrosis virus in Up-to-Date, but, as pointed out in the text, the evidence in support of this conclusion is capable of another interpretation; in any case the co-existence of two or more strains of X in potato plants is apparently quite common in Nature (19).

The conclusions reached are not in accordance with those of other investigators, who took the view that Up-to-Date contains two distinct viruses, virus X responsible for the mosaic symptoms in *Datura Stramonium,* tobacco, etc., and the top-necrosis virus which Bawden (2) designated B. Bawden found that the thermal inactivation point of the top-necrosis virus was identical with that of X, and this property has, up to the present, proved to be a very reliable index of relationship between viruses. Bawden's main reasons for concluding that the top-necrosis virus was distinct from X were (*a*) because he could isolate a non-necrotic X from Up-to-Date, and (*b*) because X failed to confer immunity against B. The isolation of a non-necrotic X is not proof that the top-necrosis virus is not also a form of X, and his evidence therefore rests entirely on the absence of cross-immunity between the two viruses, which has not been confirmed in the present investigation.

The strongest reason for regarding B as a virus distinct from X was advanced by Dykstra (11) when he stated that B could infect Seedling 41956, which has been repeatedly shown to be immune to X. In the present work it was found impossible to infect 41956 with any virus from Up-to-Date, either by top-grafting, core-grafting, or sap inoculation. It would be possible to explain the discrepancy between the two sets of results if it could be assumed that all Dykstra's infections of 41956 were carried out by the double-grafting method, as described in his second report (12). It has been shown that when Up-to-Date is double-grafted on 41956 and a susceptible basal stock, the virus (or viruses) present in the Up-to-Date passes unchanged through the 41956, but does not infect it; the same holds true when President infected with virus X is used as an upper scion. In other words, no resistance is offered to the passage of virus X or the Up-to-Date virus by X-immune Seedling 41956 when the latter is inserted as an intermediate scion between two susceptible varieties. To prove the absence of X in his Arran Victory basal stocks, Dykstra inoculated from the necrotic foliage to *Datura Stramonium* and pepper, which showed no symptoms. However, Smith (23) recorded in 1928 that inoculation from necrotic shoots of Arran Victory potato which had been grafted with Up-to-Date failed to produce symptoms in tomato plants, and it is the writer's experience that

sap inoculations from plants showing top-necrosis may be successful, but more frequently yield negative results, even though the responsible virus may be one which is easily sap-transmissible. For this reason one might suggest that Dykstra reached an erroneous conclusion regarding the virus content of his basal stocks by relying on the negative results of tests with necrotic foliage. It must be pointed out, however, that in his preliminary report (11) Dykstra specifically states that virus B was conveyed to 41956 by top-graft, and then to tomato by sub-graft from 41956.

Apart from the identification of B as a strain of X, the behaviour of Seedling 41956 in allowing the passage of virus X through its tissues in the double-grafts, without itself becoming infected, is interesting. Clearly, virus X is unable to multiply in 41956, although other potato viruses can do so. The reason for this is unknown, but assuming that the virus is a chemical substance of protein nature (3), it may be that some ingredient or physical condition essential for its synthesis is lacking in the cells of this variety. It appears unlikely that the immunity of 41956 to virus X is due to destruction of the virus by antibodies or other toxins present in the tissues. If such substances are present at all (and it has been found impossible to demonstrate them in vitro) they cannot be operative to any extent in the stem in view of the fact that virus X can survive passage through 12–14 ins. of immune Seedling stem in the double-grafted plants. If virus X does not multiply in Seedling 41956, it follows that its movement through the tissues of this variety is not the result of an autocatylitic reaction; it seems probable that the particles of virus X are merely carried passively downwards from the upper scion through the Seedling 41956 into the susceptible basal stocks, where they multiply freely. The same applies to the top-necrosis virus from Up-to-Date. There is no reason for not believing that this is also the mode of transport of these viruses in the stems of susceptible varieties; and it is noteworthy that the time taken for the development of symptoms in the basal stocks of double-grafted plants was of the same order as in single-grafted plants. Furthermore, it has been pointed out in the present paper that where food translocation from an Up-to-Date scion appeared to be impeded, no virus passed into the basal Arran Crest stock, although the scion remained alive. Clinch and Loughnane (6) previously noted that when a small tuber formed at the base of an Up-to-Date scion grafted on healthy President potato, no disease appeared in the stock plants during a period of seven weeks following grafting. Removal of the tuber, however, with the consequent diversion of the food stream from the tuber reservoir into the President stem, resulted in the development of top-necrosis 10 days later. This indicates that the downward passage of the Up-to-Date virus in grafted plants is directly connected with the movement of elaborated sap. In this connection a recent review by Bennett (4) of the evidence indicating a correlation between virus movement and food translocation is of considerable interest.

Dennis (10), as well as Dykstra, claimed to have infected 41956 with the top-necrosis virus from Up-to-Date (*Solanum virum* 4), and by this means to

have freed it from the X virus believed to accompany it. As already mentioned, the virus thus isolated by Dennis produced extremely severe symptoms of the X type in *Datura Stramonium*, tomato, and several other solanaceous hosts. In physical properties, as well as serologically, it was found to be identical with X; the two viruses, however, were not mutually protective. Dennis's results are therefore completely at variance with those of the present author who has so far failed to infect X-immune Seedling 41956 with any virus from Up-to-Date, or with any virus resembling X.

The author is indebted to Dr. R. McKay, University Lecturer in Plant Pathology, and Mr. J. B. Loughnane, with whom various points in connection with this work have been discussed. Grateful acknowledgment is also made to Professor W. J. Kearney, M.R.C.V.S., for his valuable help in performing the rabbit injection experiments.

Summary.

Of 39 varieties of potato which have been tested, the majority react with top-necrosis when grafted with scions from "streak"-carrying Up-to-Date potato. The remainder show a transient mottle similar to that in Up-to-Date itself, excepting Irish Chieftain, which carries virus A, and which reacts with crinkle (X + A).

Sap inoculation of the "streak" virus (syns. virus B, *Solanum virus* 4) from Up-to-Date to healthy potato plants of intolerant varieties results in local necrotic lesions, but the necrosis rarely becomes systemic. The virus is easily transmitted to potato plants of tolerant varieties.

Sap inoculation from Up-to-Date to *Datura Stramonium*, tobacco and tomato, as well as to *Lamium hybridum* (Order *Labiatae*), results in mild mosaic symptoms of the type characteristic of virus X; the presence of the "streak" virus in the infected plants was demonstrated by return grafts to potato.

All attempts to infect X-immune Seedling 41956 with a virus from Up-to-Date, or with virus X, were unsuccessful, but the variety was readily infected with potato viruses unrelated to X.

When Seedling 41956 was inserted as an intermediate scion between Up-to-Date and a susceptible basal stock in double-grafted plants, the virus content of Up-to-Date passed unchanged into the basal stocks. In similar double grafts in which the Up-to-Date top scion was replaced by X-infected President, no resistance was offered by the intermediate 41956 scion to the downward passage of X. The 41956 scions themselves failed to become infected with X, or with any virus from Up-to-Date.

Removal of X from Up-to-Date sap by precipitation with anti-X rabbit serum resulted in a simultaneous removal of the virus responsible for "streak."

The physical properties of the Up-to-Date "streak" virus are similar to those recorded for viruses of the X type.

The "streak" virus could not be introduced by sap inoculation to potato or *Datura Stramonium* plants already infected with virus X. The immunity of X-infected plants to the "streak" virus or to other strains of X does not hold if the second virus be introduced by graft.

Following inoculation with "streak" Up-to-Date sap, healthy potato plants of intolerant varieties occasionally developed systemic infection with a non-necrotic X virus, whereas similar plants inoculated with a mixture of "streak" sap and X-infected sap all developed systemic X infection. The possibility that in the former case the non-necrotic X may develop as a variant of the Up-to-Date "streak" virus in the local lesions is suggested.

The conclusion has been reached that the "streak" virus (virus B, *Solanum virus* 4) in Up-to-Date potato is a strain of virus X, and differs from typical X only in its effects on certain varieties of potato. Whether or not a non-necrotic strain of X co-exists with the top-necrosis strain in Up-to-Date is not certain.

The nature of the immunity of Seedling 41956 to virus X, and the probable mode of transport of X in potato stems is discussed in the light of the present results. A correlation between movement of the Up-to-Date virus and food translocation is indicated.

EXPLANATION OF PLATE I.

FIG. 1.—Leaf of healthy British Queen plant showing local lesions as a result of inoculation with sap from "streak"-carrying Up-to-Date potato.

FIG. 2.—Leaf of *Datura Stramonium* plant, showing mosaic symptoms following inoculation with Up-to-Date sap.

FIG. 3.—Double-grafted potato plant showing A upper scion of Up-to-Date, B intermediate scion of X-immune Seedling 41956, and C basal stock of British Queen. Note large, vigorous branch of 41956 scion, and top-necrosis in several shoots of basal British Queen stock.

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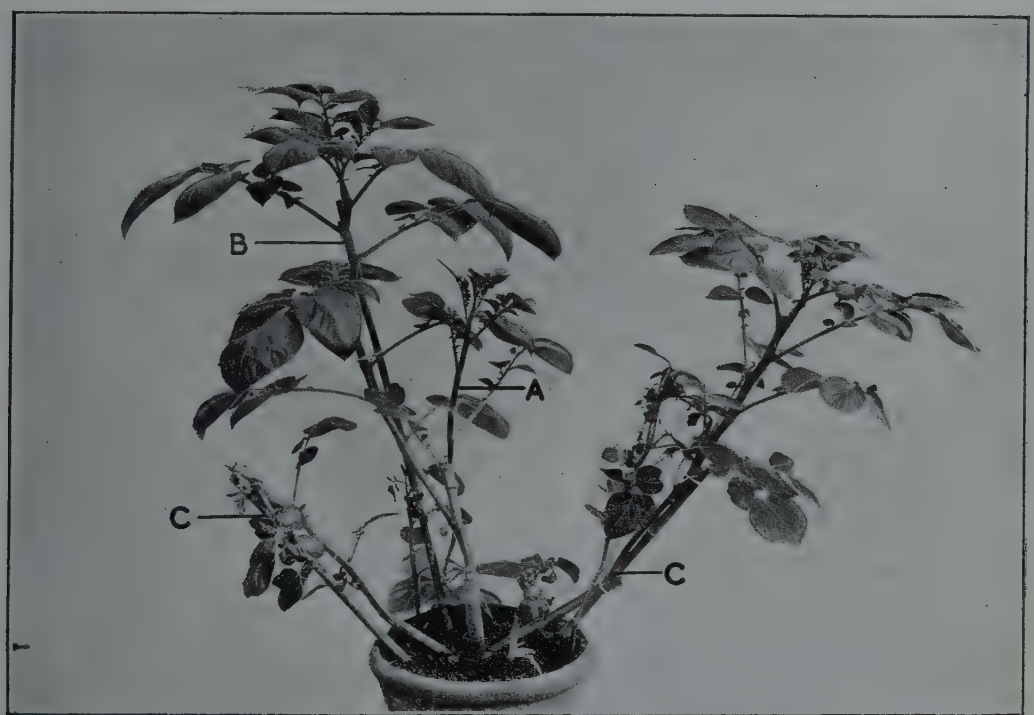
FIG. 1.

FIG. 2.



Photographs FIG. 3. *G. H. McLean.*

No. 5

FORMATION OF GYNOSPORE, FEMALE GAMETOPHYTE, AND ARCHEGONIA IN *SEQUOIA*.

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[Read June 23. Published separately November 4, 1942.]

Two species of *Sequoia* are commonly recognized—*Sequoia gigantea* (the Big Tree or Sierra Redwood) and *Sequoia sempervirens* (the Redwood or Coast Redwood). In spite of their intrinsic interest our knowledge of their life-histories is still incomplete. Earlier observations were made by Shaw (1896), Arnoldi (1899a, 1899b, 1900), and Lawson (1904), but these are either incomplete, conflicting, or at places inaccurate. More recently fertilization and proembryo formation were described for the first time in *S. gigantea* by Looby and Doyle (1937), who also revised and corrected Lawson's account of these phases in *S. sempervirens*, while Buchholz (1939 a, b) described the course of the embryogeny in both cases. It is clear from these recent papers that the two differ markedly in proembryo and embryogeny type. Buchholz (1939 c) has made further comparisons between them, and comes to the conclusion that these differences between them are so great as to necessitate the re-establishment of a separate genus for *S. gigantea*.

Details of the earlier development, from the first appearance of the gynospore to the mature gametophyte, are still uncertain in many features, and it is the purpose of this paper to complete this phase of their life-history, and to revise, if necessary to correct, any accounts already available of these stages.

Sequoia sempervirens.

Gynospore formation. Both Shaw (1896) and Lawson (1904) refer to this stage but their accounts do not agree, except that both record the occurrence of several spore mother cells. Thus Shaw states that each mother cell divides twice giving tetrad groups, of which two are figured in his Pl. **24**, 5*f*. Lawson, on the other hand, specifically states that "in no case was there any evidence of four spores having been formed from a single mother cell." His account of what he considered to happen is not clear, nor are his figures (Pl. **1**, 9, 10) enlightening. It is not certain whether he wishes to state that only one division occurs in a mother cell, or that only three spores are formed from each, nor does he figure any real stages in spore formation. It was thus necessary to revise this stage thoroughly.

The earliest stage—mid-December—at which spore mother cells are even faintly discernible is drawn in Fig. 1*a*. It is possible, as Shaw describes, that this faint group may primarily have had a hypodermal origin, but sections of younger ovules could not be so interpreted. At this early date the integument

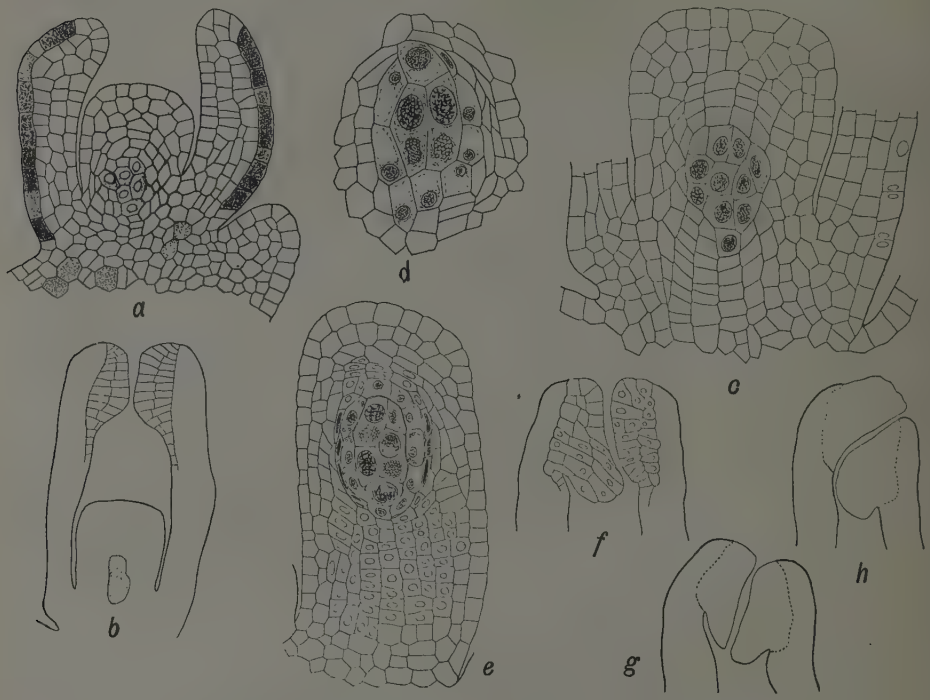

Fig. 1.—*Sequoia sempervirens*. *a*. Ovule in L.S. to show very young archesporium. × 150. Dec. 15. *b*. General plan of older ovule to show position of maturing archesporium, upgrowth of integument and young closing cells. × 92. Feb. 16. *c*. Detail of nucellus with maturing spore mother cells. × 200. Feb. 16. *d*. Two mature gynospore mother cells with sterile ones forming a one-layered tapetum. × 233. Mar. 17. *e*. Detail of nucellus to show clump of spore mother cells in early prophase, tapetum, and active cell division below. × 150. Mar. 17. *f, g, h*. The three types of micropile closure described in text. × 67. Mar. 17.

begins to elongate, as recorded by Shaw, and many ovules may be found with a micropilar tube longer than the nucellus. Development is slow, the next stage figured being two months later. The general outline at this stage is shown in Fig. 1*b*, the details of the nucellus and the maturing mother cells in Fig. 1*c*. Pollination is effected at the end of January or early in February, and the top of the long neck has already begun to close. The clump of mother cells is now quite distinct, although their number may vary from many to few. Some

few divisions have taken place in the nucellus above them, and already activity is appearing in the cells below. This latter feature becomes increasingly important later. By mid-March the mother cells are mature and preparing to divide, a date which agrees with Shaw's record for spore formation in Californian material. At this stage a peculiar feature is shown by the closing cells of the micropile. Elongation of these cells is seldom uniform round the micropile, but usually along two sides to form a slit. Along one side, Fig. 1*f*, active expansion of the lower cells produces a downwardly directed flap. Occasionally this may be formed on both sides, Fig. 1*g*, or, rarely, the type shown in Fig. 1*h* may occur. Ovules may be rather frequently found with two or even three nucelli, the whole roofed over by larger micropilar growth of one of these types. In all of these cases, of course, closure is initiated by elongation and division in the inner epidermal cells of the upper micropilar region, as in the ordinary type of closure.

The mother cells are commonly numerous. As they mature the outer ones lag behind, and form a definite tapetal zone around the functioning ones. Details of the nucellus, the arrangement of the tapetal layer, and a typical group of mature mother cells in early prophase are shown in Fig. 1*e*. The tapetal cells rapidly become vacuolate. It is possible that the tapetal zone may, in some cases, be completed by contiguous nucellar cells, but it is, in the main, derived from the archesporium. The functioning mother cells are large, and show a massed clump of starch grains below the nucleus. The number of these cells may vary, depending obviously on the number organized in the archesporium. Fig. 1*d* shows a slightly younger stage with only two. There may occasionally be only one functioning mother cell, and cases have been met in which only one or two were present with no surrounding tapetum, clearly cases in which the archesporium was composed of only a few cells. The ovule from which Shaw's Pl. **24**, 2 was drawn might well have been of this type; but Fig. 1*e* is the commonest condition. This figure also shows the initiation of active and very regular divisions in the nucellus below.

The shape of the mother cells varies, and with it the distribution of the starch during division. Some are elongated, with a well-defined starch zone below—if only two or three are present they are usually of this form. If many are present some at least are polyhedral, with a more uniform starch distribution. This is reflected in the first divisions drawn in Fig. 2*a, b, c*, chosen, at random, just to show the fact that the lie of the spindle varies. The division may result in a transverse wall dividing two cells, one with all the starch grains, the other with none; or in two cells with an equal starch content more uniformly distributed in each; or in a number of intermediate types. A second division follows in each, resulting in groups of four, these tetrads varying greatly in their appearance and arrangement. These second divisions were not met with as frequently as the first, and this perhaps accounts for Lawson not finding them, although they had already been recorded by Shaw. Two examples of these second divisions are drawn in Fig. 2*d, e*. These two

were selected because they showed well the two divisions in the plane of the section. The two divisions may, however, be at right angles, or at any angle, to one another. A series of the resulting tetrads are shown in Fig. 2*f* to *o*,

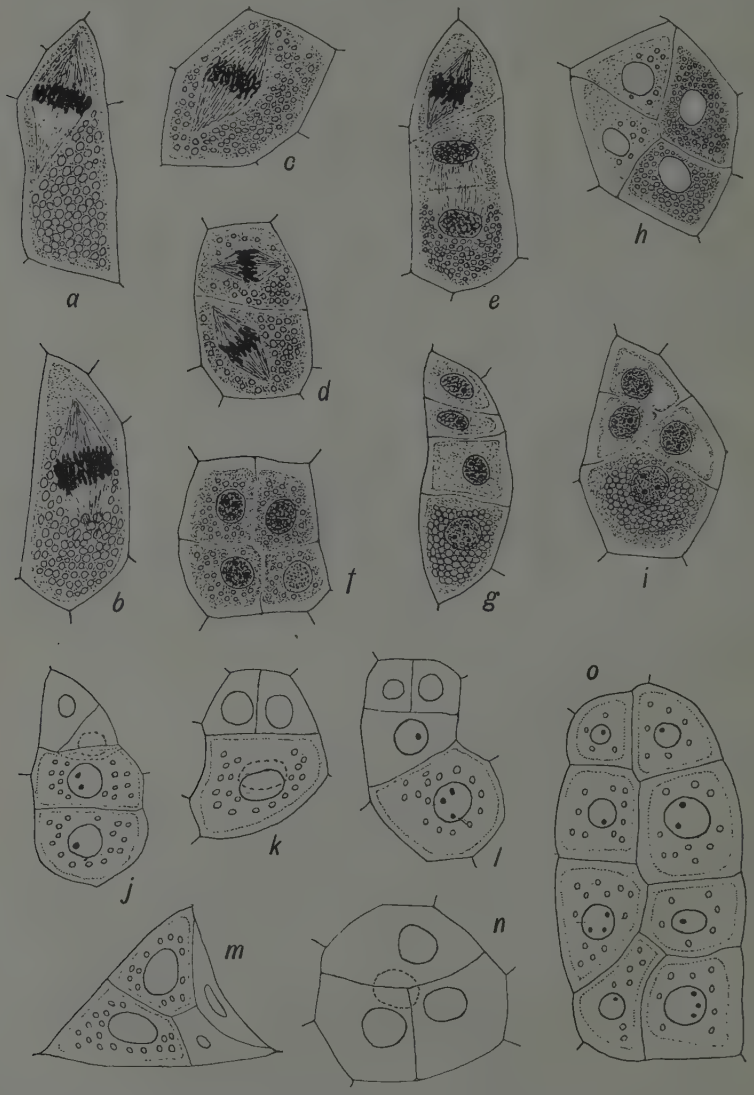

Fig. 2.—*Sequoia sempervirens*. *a*, *b*, *c*. First division in three spore mother cells. × 725. Mar. 25. *d*, *e*. Two second divisions. × 625. Mar. 25. *f*–*o*. Examples of tetrads referred to in text. × 625. Mar. 25 to Apr. 1.

some in detail, some in plan. They may be symmetrical, like pollen tetrads—in one plane, Fig. 2*f*; or tetrahedral, Fig. 2*n*; or with two of the cells having more starch and denser protoplasm than the other two, Fig. 2*h*. They may be unsymmetrical—with two small starch-free cells above, side by side, and two larger starch-filled ones below, either side by side, Fig. 2*k*, or superimposed, Fig. 2*j*, or a variant of this latter resulting from an oblique division in one pair, Fig. 2*l*; or showing only one large cell with starch and three small upper ones, the whole obviously resulting from two oblique divisions, Fig. 2*i*. Typical linear tetrads may occur, Fig. 2*g*. The arrangement shown in Fig. 2*o*, two linear tetrads with four large spores in each, was only met with once. As no other spore tetrads accompanied these they were probably derived from an archesporium with only two functioning mother cells originally, as in Fig. 1*d*. This example recalls the only tetrad drawing published by Shaw.

Details of the nucellus with tetrads *in situ* surrounded by the tapetum are shown in Fig. 3*a*. It was difficult to make a satisfactory drawing at this stage. The mother cells remain in contact, not rounding off as in pollen development, but their walls are very thin and usually do not stain at all or only very poorly; the membranes cleaving the tetrads are more delicate still. If a perfect fixative could be used the tetrad effect would not be so outstanding, but, luckily, the spores are hard to fix without contraction, and on contraction they tend to show the protoplasmic contents bunched round a central point or plane, the appearance naturally varying with the original shape of the tetrad. Thus when the walls do not stain there appear to be widely separated and distinct tetrad groups scattered loosely through the space enclosed by the tapetum, somewhat as shown in the general plan in Fig. 3*b*. The tapetum is still distinct, but already shows signs of degeneration, more particularly below the spore area. From the time of its first formation it shows no increased development but a gradual senescence till it is completely disrupted later. During this period, also, cell division in the nucellus *below* this area becomes very active, resulting in the appearance shown in Fig. 3*b* recalling the structure in the young sporophyte of *Pellia*. In the continued course of this meristematic activity there develops a difference between a central zone of somewhat larger and more cubical cells and an outer zone of more compact tissue with narrower cells brick-like in shape. This is indicated in some of the later drawings, for instance, Fig. 3*e*. Down through this central zone the growing prothalli penetrate.

In transverse section, when a large number of symmetrical tetrads are present, the resemblance to a section of a young eusporangium is striking.

Development of female gametophyte and archegonia. The accounts given by Shaw and Lawson of the earliest stages in the development of the prothallus agree, and are correct enough, but, as neither author presents satisfactory figures, it was thought advisable to include for completeness a few drawings. Clearly there may be from one to four potentially functioning spores in each of several

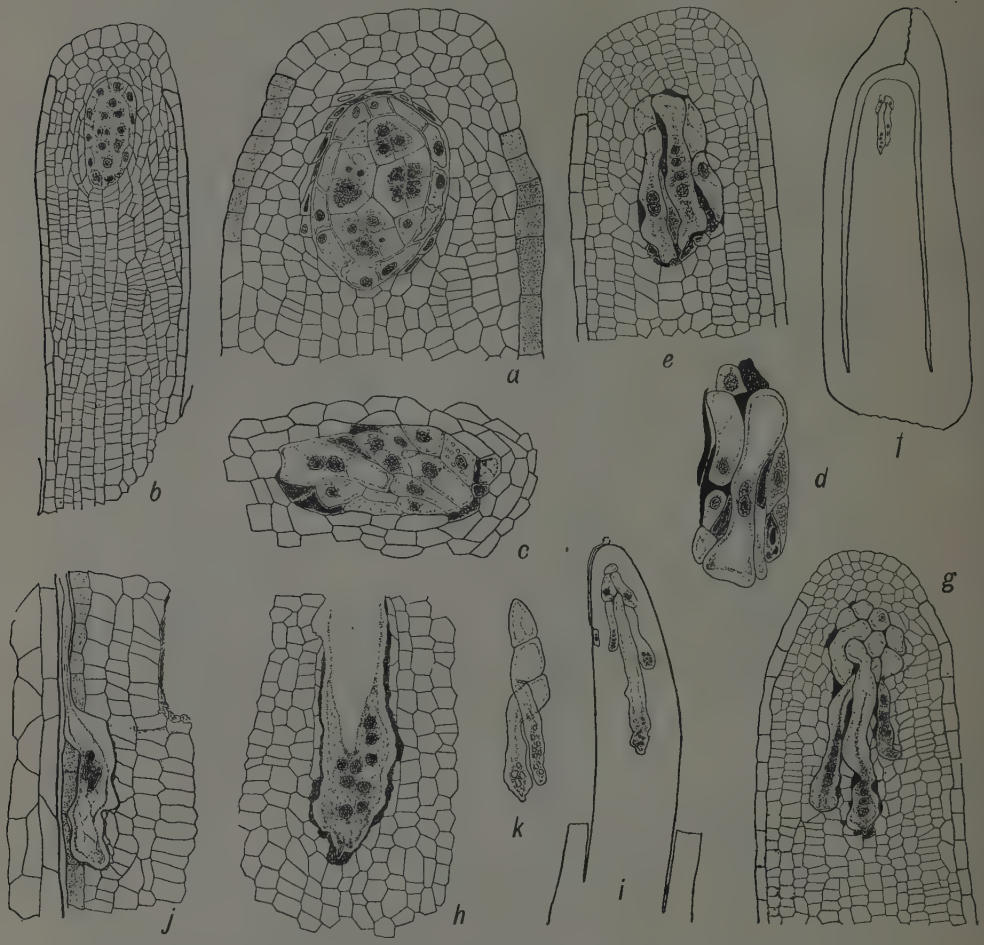

FIG. 3.—*Sequoia sempervirens.* *a.* Detail of nucellar apex to show tetrads *in situ* with surrounding tapetum and the deep-staining epidermal zone. × 200. Apr. 1. *b.* General plan of whole nucellus to show nature of the active cell division. × 92. Apr. 1. *c.* Group of enlarging spores, young binucleate stages and degenerating cells. × 200. Apr. 15. This fig. lies at right angles to the position in the ovule. *d.* Older binucleate gametophyte showing also young stages of spore enlargement, degenerating cells and spores (top and at right with starch grains) which have not yet degenerated. × 200. Apr. 30. *e.* A young four-nucleate gametophyte and a binucleate one both beginning to bore down through the central zone of slightly larger cells; general arrangement of nucellar tissue above and the deep-staining epidermal layer also shown; tapetum practically disintegrated. × 100. May 10. *f.* Plan of ovule to show general proportions at the 4-nucleate gametophyte stage. × 30. May 10. *g.* Group of 4-nucleate gametophytes growing down together, the central one showing the nuclei below and the boring tip. × 107. May 15. *h.* Tip of 16-nucleate gametophyte. × 107. May 24. *i.* General plan of ovule to show proportions at this stage and also location of lateral entry of pollen tube. × 30. May 24. *j.* Detail of lateral entry of young pollen tube, growing between the nucellus and the integument (on the left) and penetrating the special epidermal layer of the nucellus. × 200. May 24. *k.* Plan to show growth of two young prothalli from a single linear tetrad. × 67. May 24.

tetrads. Most, if not all, of these enlarge a little, and become vacuolate. At this stage the tetrad grouping becomes rapidly lost, as some of the spores advance quickly, the others, the majority, slowly or not at all. This results in the appearance shown in Fig. 3*c*. Only crushed traces of the tapetum are now left, and the small non-functioning spores in many of the tetrad groups degenerate also. In some of the spores the first division has already produced young binucleate prothalli, of which quite a number will continue further growth for some time. The two nuclei at this stage lie centrally—a slightly older binucleate prothallus is shown in Fig. 3*d*. The second division takes place while the nuclei are still central—Fig. 3*e*—but the four nuclei pass to the tip, which bores actively down through the central part of the nucellus. In Fig. 3*g* three of these four-nucleate gametophytes are shown, two of them being about equal in growth rate. Two others of similar size were present in the ovule from which these were drawn. Above and laterally can be seen the remains of earlier stages which will not develop further, as well as vacuolate spores and some spores already degenerating. The general proportions of these gametophytes to the whole nucellus are shown in the sketch plan, Fig. 3*f*. Fig. 3*k* is from an ovule which developed only one tetrad, which is giving rise to two gametophytes. These two figures help to emphasize the peculiar feature of this prothallial growth, boring downwards like a thick pollen tube, as well as the relative distance still to be traversed. Simultaneous divisions of the nuclei continue as the successful prothalli push downwards, but usually one is much in advance of the others at the 16-nucleate stage, and will ultimately produce the functioning gametophyte. The details of the growing apex of such a 16-nucleate one are shown in Fig. 3*h*, and its relations to the nucellus at this stage in Fig. 3*i*. The burrowing tip is a feature of interest.

If the dates of the stages so far recorded be examined it will be seen that the tetrad formation is carried out rapidly, a common phenomenon in conifers. The mother cells take approximately three months to mature, but a period of fifteen days or less covers tetrad formation in almost all ovules. It is a feature in *S. sempervirens*, noted by all who have examined it, that at any given date considerable variation will be found in the stages shown by different ovules and in different cones. But the fifteen-day period of tetrad formation covers all except extreme or abnormal cases. Gametophyte development up to the 16-nucleate stage takes from six weeks to two months. The relative rapidity with which the tetrad stages are completed may perhaps account for Lawson overlooking these very interesting phases.

The pollen grains have in the meantime germinated. The growth of pollen tubes over the top of the nucellus and down its side between it and the integument is well known from Lawson's figure, Pl. 1, 3. Lawson, however, does not figure the lateral penetration of the nucellus by such tubes, nor indicate either the date or the location of penetration; indeed, although he draws (Pl. 1, 4) an example of direct entry of a pollen tube into the top of the nucellus he does not specifically mention that the lateral tubes do actually

penetrate the side of the nucellus, although that can readily be inferred. Shaw also describes lateral pollen tubes, and states that the penetration is lateral, but again gives no figure. He obviously, from his own account, had difficulty in following certain stages. Further, the structure of the nucellus suggests that its upper surface is a receptive area, the epidermis of the sides becoming increasingly distinct and staining more deeply as the ovule matures. But this forbidding-looking epidermis actually presents no obstacle to many of the tubes, the lateral penetration of one of them being shown in plan at the top of Fig. 3*i*, and in detail in Fig. 3*j*. In the latter figure is seen the deeply staining epidermal zone through which the tube has only recently made its way. In the tube the body nucleus and the two sterile nuclei are still bunched together in the same mass of cytoplasm. A stage such as this can be found from the latter part of May to well into June. Penetration of the upper surface of the nucellus may also occur. Shaw records branching of the tube. Lawson says this does not occur; nor was it seen in any of the ovules here examined.

The gametophyte, as it grows down, expands at the expense of the nucellus—later only the epidermis of the nucellus may be left at places as shown in Fig. 4*a*. As it grows, continued division takes place in the nuclei, many of which are left in the upper part, although the lower zone is still dense and multinucleate. And so is formed the peculiar gametophyte, well known from the descriptions of Arnoldi and Lawson, showing the upper vacuolate zone with parietal protoplasm and a single layer of nuclei, and the lower dense continuous free-nuclear zone. Arnoldi describes the occurrence of a small free-nuclear area at the top of the gametophyte. This has been met with several times. It is probably of no great importance, as a similar small area can be seen in other conifers, *Callitris* spp. and *Wellingtonia* (*Sequoia gigantea*) for example, where it is found when the apex is pointed and narrow, and the normal parietal protoplasm becomes condensed for lack of room. The pollen tube in the meantime has grown in to make contact with the gynospore membrane. It grows down the side of the gametophyte, sometimes straight, sometimes obliquely, but always outside the gynospore membrane. It may press into the prothallus, forming a shallow groove, but above the free-nuclear zone it becomes fixed by the formation of a large hemispherical invagination—Figs. 4*d* and 5*e*. The tube then contracts, and continues for a short distance further down. This type of tube also occurs in *Wellingtonia*, and has already been described by Looby and Doyle (1937) in both species. Lawson, however, says that the pollen tube may sometimes be embedded in the gametophyte, Arnoldi, on the other hand, stating that it always grows down between the nucellar tissue and the spore membrane. As noted in the earlier paper (Looby and Doyle, 1939) and as now further confirmed by a large number of early developmental stages, Arnoldi's observations are correct. It is possible that it may appear at places as if embedded, but any such apparent embedding must doubtless be explained as in the similar case of *Callitris*. According to Saxton (references in Looby and Doyle, 1940) the pollen tube in the callitrinean conifers

(*Callitris, Widdringtonia, Actinostrobus*) penetrates the gametophyte even before cellular formation begins; but, as shown by Looby and Doyle (1940), this is definitely not the case in *Callitris* and probably not in the other genera. It was there shown that the apparent embedding was due to the growth of gametophytic tissue round the sides of the lateral pollen tube which is always morphologically outside the membrane.

Cell formation and archegonium development have been described by Arnoldi and Lawson. As these two accounts differ radically it was necessary to clarify these phases. Thus, in general, Arnoldi claimed that, while free cell formation takes place in the lower zone of the gametophyte, yet typical alveoli are formed in the vacuolate area; that only in this alveolate zone are archegonia formed; and that each archegonium is formed "aus einer einzigen peripherischen Endospermzelle." Admittedly his figures are not very convincing. Lawson claims that free cell formation takes place throughout; that the alveoli described by Arnoldi are merely longer cells in the midde region; and that the archegonia arise from deepseated clumps of cells, and grow or expand outwards till they reach the periphery or make contact with the pollen tube. These two latter points are illustrated in his Pl. 3, 26 and Pl. 2, 17. Actually again Arnoldi's observations are correct. Buchholz (1939b), although he did not intensively study the archegonial origin, has made some interesting criticisms of Lawson's statement, which he considers probably erroneous. These suggestions of Buchholz are quite borne out by a closer examination. It is clear that Lawson did not realize the importance of the relations of the pollen tube and its invaginated bulge to gametophyte development and the origin of the functioning archegonia. Thus his drawing (Pl. 2, 17) is taken from the upper part of the gametophyte, shows nothing of the location of the pollen tube, and ignores the fact that the male cells and the functioning archegonia are much farther down.

In the first place, then, typical alveoli are formed all along the vacuolate area. These originate and develop in the manner originally described by Sokolowa (1890) and to which attention has been recently redirected in the account of alveolar formation in *Callitris* (Looby and Doyle, 1941). [*Callitris*, and probably also *Actinostrobus*, actually differ a little from the type, but merely in the preliminary formation of the outer single layer of small cells on which the alveoli are based.] Numerous stages in the development of these alveoli are available, but they repeat so closely the typical form that it seems unnecessary to reproduce them. The drawing, Fig. 4*a*, should suffice to show that, down even to the pollen tube bulge and to the position of the body cell, typical alveoli have been formed, and that the archegonial initials are merely modified alveoli. Lower down free cell formation takes place in the free nuclear region, resulting in the very different appearance shown in Fig. 4*b*. Sections across the upper and lower regions at this stage are quite unmistakable. Above the pollen tube bulge comparatively few alveoli become complete archegonia, and these mostly facing and close to the tube. But, as mentioned by Looby and Doyle (1937) and Buchholz (1939b), they may develop, both in this region and

also at the bulge level, facing the periphery anywhere at the surface of the gametophyte. Imperfect archegonia are common, especially above the bulge. Alveoli, instead of forming a neck and an egg, may segment transversely to their own long axis—this is the early stage of normal cell formation in this region—but this may be done irregularly, and may result in rows of cells of different sizes, of which some are large and develop denser protoplasm. There can be found all possible grades between normal cell formation, true archegonia, and rows of jacket-like cells. In some cases larger cells may be separated from

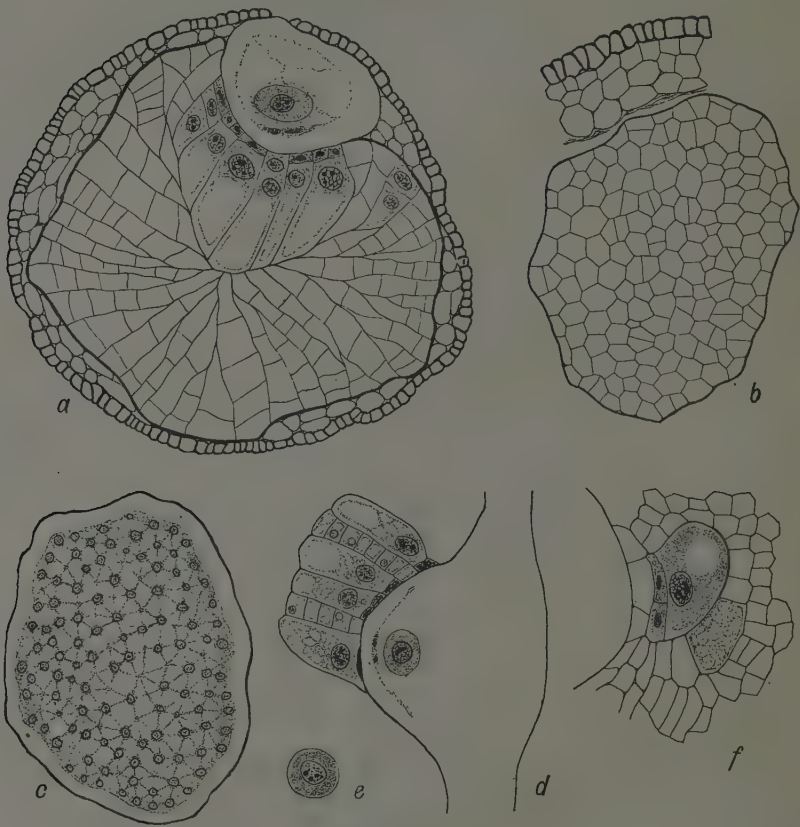

FIG. 4.—*Sequoia sempervirens.* *a.* T.S. nucellus and contained gametophytes through the pollen tube bulge, to show alveoli based on gynospore membrane, the thick line; alveolar origin of archegonia; and pollen tube, with body cell and sterile nuclei, *outside* the membrane × 100. Aug. 3. *b.* T.S. through lower free-cellular zone. × 115. Aug. 3. *c.* T.S. young gametophyte through the free nuclear zone. × 100. July 30. *d.* L.S. pollen tube along invagination area to show archegonia towards top of the bulge and body cell in tube. × 115. Aug. 6. *e.* Male cell. × 125. Aug. 10. *f.* Archegonium separated from pollen tube by a layer of cells. × 138. Aug. 10.

the exterior by other smaller cells, in one or more of which, occasionally, anticlinal division may take place; the result then resembles the drawing given by Lawson of the archegonia in the upper part of the prothallus. More particularly, if numerous well formed archegonia are present in a longitudinal section cut obliquely it is very easy to misinterpret the structure shown. In fact every alveolus is potentially fertile; some remain vegetative; some form archegonia; some develop abnormally. Frequently in the more abnormal cases masses of large deeply placed cells distributed irregularly or compactly may be seen—these probably explain Lawson's drawing, Pl. 3, 26. At the pollen tube bulge the archegonia are usually more compactly arranged, and may be in immediate contact with one another, or separated by cell rows with walls periclinal to the tube surface—Fig. 4*d*; and here, too, abnormalities may occur. The commonest special modification of the alveoli here, however, is the formation of imperfect rows of jacket cells on the outer sides of the rather irregular complexes.

But there is one area which raises difficulties, the area round the lower part of the pollen tube bulge where the primarily vacuolate zone passes into the free nuclear part below. Examination, however, of many ovules shows that in most cases the archegonia are located in contact with the upper slope of the bulge—Figs. 4*d* and 5*e*. This area is always alveolar in origin. The lower part of the bulge, however, requires further consideration, as archegonia may sometimes be found in a lower position than is indicated in Fig. 4*d*. The stage just before alveolar and free cell formation is outlined in Fig. 5*a* for the area including the tube bulge and the transition zone from the parietal nuclear to the free nuclear stage. It is seen that the bulge, except its very lowest part, is in contact with the vacuolate zone and a single layer of parietal nuclei. The transition from the vacuolate to the free nuclear area is abrupt, but occurs more rapidly on the side away from the pollen tube. Thus when on this side there is already a double layer of nuclei there is still only a single layer in contact with the tube. A transverse section across this line is drawn in Fig. 5*b*. The protoplasmic lining may show a double layer of nuclei practically all the way round except in immediate contact with the tube. It will be seen incidentally that the protoplasm as it widens develops a series of small vacuoles, which are very characteristic of the free nuclear zone below, as shown in Fig. 4*c*. Already in this case phragmoplastic fibres (or some form of spindle) are being organized between the nuclei—this precedes membrane formation, which occurs a little sooner in this zone than higher up. It is, anyway, apparent that only well down along the lower slope of the bulge is a double nuclear layer first met with. The immediate result of membrane formation is outlined in the plan drawing, Fig. 5*c*. Two things must be remembered. Firstly, wherever there is a free nuclear condition in conifer gametophytes free cell formation takes place. Even if the layer of nuclei is only two deep the outer nuclei will become enclosed in the first complete cell membranes. Secondly, any layer of nuclei which immediately

abuts on the vacuole will proceed to form alveoli. In this drawing the small compact free cellular tissue, with continuous cell outlines, can thus be readily followed, as well as the general lie, diagrammatic of course, of the alveoli growing in from all sides and up from below. In each of these alveoli its own first division membrane is indicated by a dotted line. When tissue formation is well advanced, by the time of fertilization for example, it is obvious

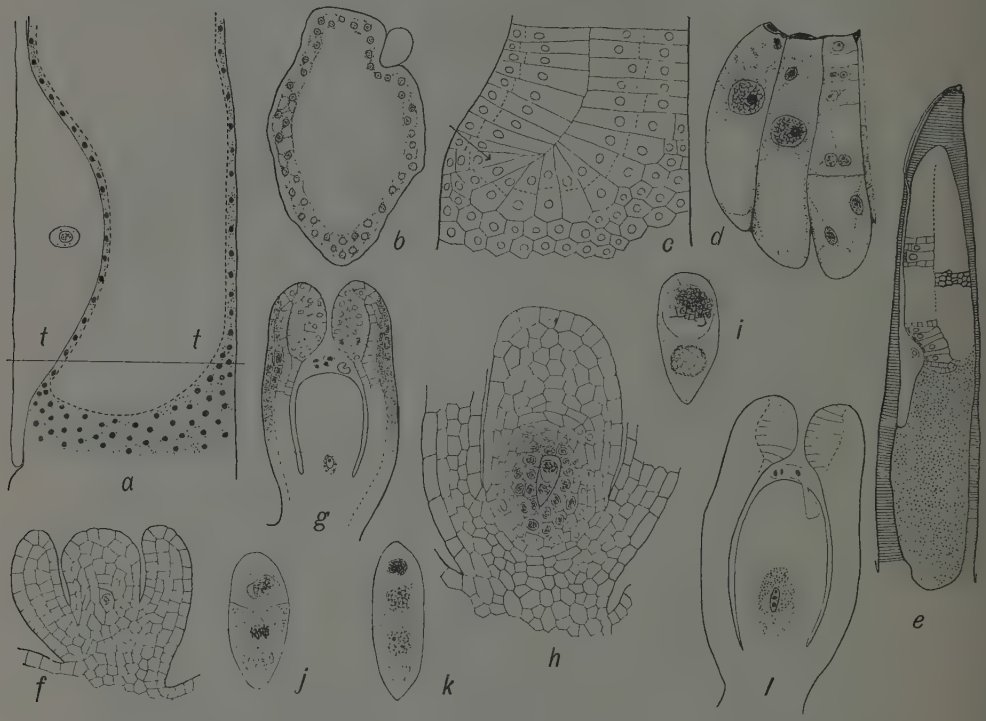

Fig. 5.—*Sequoia sempervirens.* *a.* L.s. of gametophyte to show in plan the transition from the vacuolate to the free-nuclear zone, and their relations to the pollen tube bulge. × 67. July 30. *b.* T.S. along the line *t–t* to show the single layer of nuclei opposite the pollen tube and a double layer elsewhere. × 67. July 30. *c.* Plan of first membrane formation in *a,* description in text. × 83. *d.* Possible ventral canal nuclei in two normal archegonia, a type of irregular alveolar division on the right. × 200. Aug. 10. *e.* General plan of the relations of the gametophytes and nucellus just previous to fertilization. Aug. 11.

Wellingtonia gigantea. *f.* L.S. ovule to show position of very young mother cell. × 150. Feb. 23. *g.* Outline section of ovule with single mature mother cell surrounded by developing tapetum; detail of symmetrical closure of micropile; binucleate pollen with extine shed. × 92. Mar. 25. *h.* Detail of nucellus with single mother cell and tapetum. × 200. Mar. 25. *i.* The gynospore mother cell with nucleus in prophase and cytoplasmic body below it. × 483. Mar. 25. *j.* Second division in maturing tetrad. × 483. Apr 4. *k.* Three-celled "tetrad." × 483. Apr. 4. *l.* Outline of L.S. of ovule to show location of tetrad and tapetum and germinating pollen. × 92. Apr. 4.

that there will be a definite belt in which it would be impossible to say whether its origin is alveolar or free cellular; and the origin of archegonia low down on the tube bulge may then be doubtful, even though that origin may have been alveolar. Actually, as shown here, only a few archegonia could arise from deepseated alveoli, i.e., alveoli separated initially by cells from the pollen tube and in such a situation that they could grow out, if such a possibility were assumed, to make contact with the tube anywhere along the bulge. At the point marked by the arrow one could conceive this alveolus forming an archegonium whose neck would develop from the outer cell in the division just outlined. Such an archegonium would be separated from the pollen tube by a sterile cell. Cases of this type might occur, but would be rare, and would in no way alter the fact that the normal arrangement is the formation of archegonia from alveoli directly abutting higher up on the tube. In the course of the routine examination of all the material such cases were sought for, but only one clear case was found—Fig. 4*f*—and this in an ovule which was abnormal. The mature body cell was lodged in the lower tail of the pollen tube, and many deepseated irregular archegonium-like cells were scattered through the transitional tissue, all of which were probably formed from the short basal alveoli. No example was found of an archegonium occurring in tissue of undoubtedly free cellular origin.

It seemed necessary, even at the risk of labouring the point, to clarify this question of archegonial origin. In spite of claims in the literature of a deep-seated origin for archegonia in some genera, it would appear certain, as far at least as concerns the Taxodiaceae and the Cupressaceae, that normal functioning archegonia are always formed from alveoli which have grown in directly from the periphery, the archegonia making immediate contact with the gynospore membrane. The callitrinean genera are a partial exception, since in these the cell which will form the neck of the archegonium is apparently cut off *before* the elongation of the alveolus, and not *after* as is the usual method.

From the stage shown in Fig. 3*h* development is slow, the first walls and alveoli appearing about the last week in July. Cellular formation and the maturing of the gametophyte to the fertilization stage are rapid, fertilization taking place between 6–12 August. The general plan at this stage is drawn in outline in Fig. 5*e*. This shows the lower area compactly cellular now, the transition belt at the lower part of the pollen tube bulge, and the alveolar zone, with the functioning archegonia towards the upper part of the bulge and others scattered up along opposite the tube higher up. Even in this zone, except in the immediate vicinity of the tube, the appearance of the original alveoli may be rapidly lost, cell divisions taking place in other planes as indicated on the right. Various transverse sections about this stage may be seen in the papers by Buchholz and Looby and Doyle. The subsequent stages in fertilization, proembryo formation, and embryogeny have there been described.

A few minor points remain for comment. (*a*). Buchholz (1939b) seems uncertain concerning vacuoles in the archegonia. The archegonia are fairly typical of the taxodinean-cupressinean genera. As shown above in Fig. 4*a*

there is when young a large vacuole filling most of the egg, but later the protoplasm becomes denser, and encroaches on the vacuole, which may be obliterated or reduced to a small size. The archegonia in the uppermost zone seldom mature fully, and frequently, or commonly, retain a larger vacuole. It is natural also that the functional archegonia should be shorter than those above, as they are formed from alveoli which owing to the pollen tube bulge may be only half as long as the upper ones. (*b*). Buchholz corrects our interpretation of early embryogeny. Owing to continued sterility in the material available we have been unable to revise this point but freely accept his view. (*c*). Lawson says the supernumerary gametophytes never become cellular. Buchholz, however, reports the presence of small cellular alveoli, and such have frequently been found in this investigation; indeed, in one case, two prothalli were found with archegonia in both. (*d*). Certain other statements of Lawson's, particularly those associated with his figures, Pl. **2**, 14, 18, 19 (for details his paper can be consulted), almost certainly are the result of imperfect interpretation of material which had irregularly contracted on fixation. (*e*). Lawson records the existence of a ventral canal nucleus, but not the division forming it. Buchholz thinks the appearances described are abnormal, and that a ventral canal nucleus is not regularly formed. Certainly if formed it must be very ephemeral, although cases have been met with showing a second smaller nucleus in the archegonium, as in Fig. 5*d*, which seems more normal than the nuclei described by Buchholz. As examples such as this one are rare, and as no divisions were met with, the matter must wait for further investigation. Ventral canal nucleus formation may be vestigial here, occurring rarely and even then, as often as not, abnormally.

Wellingtonia (*Sequoia*, *Sequoiadendron*) *gigantea*.
Sierra Redwood.

Arnoldi (1900) has briefly recorded the gametophyte here, in contrast to that of the Coast Redwood, as normal, that is with alveolar growth all the way round. He also gives a few words on archegonial structure. Buchholz (1939a) has also recently recorded typical alveolar growth. No information, however, is anywhere available on gynospore origin or early gametophyte development; and, in view also of the unsatisfactory nature of the previous accounts of structure in *S. sempervirens* which necessitated the work already outlined in this paper, it seemed advisable to make a complete survey, however rapid, of all the corresponding stages in the Sierra Redwood.

Gynospore development.—A clear indication of a gynospore mother cell was first seen late in February, only one such cell being formed, Fig. 5*f*. Rarely, more than one may be formed, but in all the ovules examined never more than two were seen, and that only twice. Material collected in late December showed a smaller uniform nucellus with no trace of special cells. At this date indeed some of the ovules were so young that the integument was only starting to grow. In ovules collected in January a faint grouping of three or four cells in the mother cell position could sometimes be seen, suggesting the

presence of perhaps four potential mother cells, of which only one functions, but there was never more than a suggestion of this. It was not possible to trace this faint group or the single functioning cell to a hypodermal origin. It is, however, interesting to compare ovular size at the stage of Fig. 5*f* with the corresponding stage in *S. sempervirens* shown in Fig. 1*a*. Obviously the ovule of the latter, the ovule of the polyploid type, is much the larger.

The single mother cell matures slowly, but is ready for division about the third week of March or earlier. Structure at this stage is shown in general plan in Fig. 5*g*, the details in the nucellus in Fig. 5*h*. Pollination has recently taken place, so that micropilar closure has begun. The closing cells are typical, and can be seen to be formed by the elongation and subsequent division of a zone of epidermal cells lining the micropile. The closure is symmetrical on two sides of the opening, thus forming a slit which lies in the plane of the developing ovular wings. The special feature is the change of the nucellar cells surrounding the mother cell to form a distinct tapetum. The mother cell at a greater magnification is further shown in Fig. 5*i* to bring out the large nucleus just entering the prophase of its first division, and, particularly, the definite cytoplasmic body, which may be so large as to be mistaken for a second nucleus. The pollen grains have thrown off their extine, but have made no further development. It will be noticed also that the micropilar tube does not extend considerably beyond the nucellus (cf. Fig. 1*b*).

A very complete series is available of the formation of the three-celled "tetrad," but only a few stages are illustrated. The second division is drawn in Fig. 5*j* to show that it is the lower cell with the cytoplasmic body which divides. The "tetrad" stage is shown in Fig. 5*k*. The tapetum has become much more definite and extensive, as shown in the general plan, Fig. 5*l*, and the detailed drawing, Fig. 6*a*. Tetrad formation is completed before the end of the first week in April. It is thus clear that in this species gynospore formation is of a more normal taxodinean type and markedly different from that in *S. sempervirens*.

A complete series is also available of early changes in the pollen grain, but no features of special interest appear. At pollination the usual large tube nucleus in the main body of the grain can be seen with the small generative nucleus pressed close to the wall in a small denser lenticular zone of cytoplasm. The intine swells throwing off the extine. The grain bulges at one side to form the pollen tube, which elongates forward to enter the top of the nucellus. The tube nucleus moves forward, the generative nucleus advances a little, swells, rounds off, and divides to form the stalk and body nuclei. This stage is shown in Fig. 6*a*. Shortly after, these two nuclei follow the tube nucleus, but growth is slow, the tube tip only reaching to about the top of the developing gametophyte by the end of June.

Development of gametophyte and archegonia. The lower of the three spores is the functioning spore. It enlarges slowly and in the ordinary coniferous fashion, that is gradually upwards, the nucellar and integument cells dividing and increasing in volume with it. Just before cell formation it has, in relation

to the whole ovule, the form and extent shown by the complete prothallus in Fig. 1 of the paper by Looby and Doyle (1937). Within the gynospore membrane, thick below but thinner above, the typical parietal lining of cytoplasm, with a single layer of embedded nuclei surrounding the large central vacuole, can be followed all the way round. Buchholz (1939a) records up to 6000 nuclei at this stage, a number which is approximately correct. Owing to a difference in size of the nuclei Buchholz (1939a) raises the question whether all the divisions are simultaneous, but could not settle the point as mitotic figures were not available. Actually, although the earlier divisions may be simultaneous, the later ones are not. A wave of mitotic activity passes from

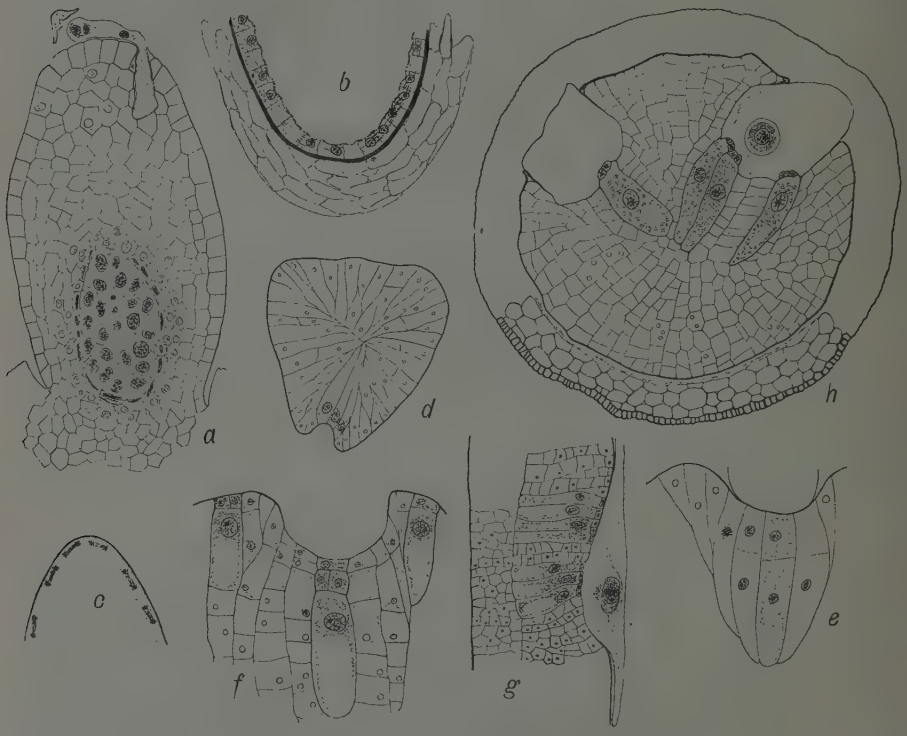

FIG. 6.—*Wellingtonia gigantea*. *a*. Detail of nucellus to show tetrad, tapetum and young pollen tube with tube nucleus passing down and the stalk-body division just over. × 200. Apr. 4. *b*. Base of gametophyte to show earliest alveolar origin from a single layer of nuclei, the thick gynospore membrane and surrounding tapetal cells. × 150. July 27. *c*. Apex of same gametophyte with nuclei only in late telophase. × 150. July 27. *d*. Very early alveoli in T.S. and very young archegonial initials. × 67. Aug. 1. *e*. Slightly older archegonia, the relatively large neck cells recently cut off. × 150. Aug. 1. *f*. T.S. of edge of gametophyte to show one type of irregular archegonium and archegonia not in contact with the tube. × 150. Aug. 18. *g*. L.S. gametophyte along pollen tube and bulge, showing alveolar origin of tissue and archegonia viewed in this plane; body cell in tube. × 67. Aug. 20. *h*. T.S. complete gametophyte and nucellus just before fertilization, showing male cell in one tube, and the protruding archegonia; further description in text. × 83. Aug. 22.

the bottom of the gametophyte to the top, as already described by Looby and Doyle (1940) for *Callitris*. A later phase, bringing out this point as well as others, is shown in Fig. 6*b* for the basal and in Fig. 6*c* for the apical area. The former figure shows that the single parietal layer of nuclei embedded in the cytoplasmic lining is definitely continued as such around the base—there is no trace of the slightest free nuclear condition. This had necessarily to be noted, as Arnoldi's Fig. 6 (1900) might be interpreted as showing a slight free nuclear development, and as Buchholz (1939a) does not figure the basal zone. This area, as shown, has already initiated the young alveoli in a typical manner. On the other hand, at the apex of the same gametophyte, as shown in Fig. 6*c*, the nuclei are still in late telophase of the last division and so appear paired with the primary spindle still between them. The additional later spindles, which will link up each nucleus with five more neighbouring nuclei just before membrane formation, as so excellently photographed in Buchholz's Fig. 16A, are not yet formed. It will also be seen that the basal area is still surrounded by the many-layered tapetum, which is a marked feature of this zone till after fertilization. It becomes thinner higher up along the sides of the gametophyte, and is only represented at the apical area by an occasional degenerating cell. It is these tapetal cells that Arnoldi has figured in his Pl. 7, 1 and described as archesporial cells. The pollen tube, in the meantime, has also been growing down in close contact with the gynospore membrane, and has taken up its final lateral position and become fixed with its characteristic invagination just before membrane formation begins.

Typical alveolar formation takes place all around the gametophyte, as already indicated by Arnoldi (1900) and Buchholz (1939a). Arnoldi merely draws the completed alveoli met in the centre. Buchholz, however, shows (his Figs. 8, 9, 10, 11) a few stages in their ingrowth. Although these figures are not quite complete, it was not considered necessary to repeat any drawings. If reference be made to the paper by Looby and Doyle (1940) on *Callitris* it would be easy to complete Buchholz's drawings by moving the alveolar nuclei in his Fig. 9 a little nearer the vacuole, and by joining the nuclei in this and in Figs. 10, 11 by a connecting spindle on which the continuous ingrowing membrane is laid down. Figs. 6*d, e* suffice to show again that the archegonia here are alveolar in origin, peripheral, and abutting directly on the gynospore membrane and pollen tube. It is, however, not easy to understand Buchholz's Fig. 12, which shows alveoli closed before they meet centrally, with the nuclei already retired back and divisions actually begun. In no case of alveolar formation that has been examined so far has anything of this nature been seen. Alveoli of the type occurring here, as in *Callitris*, grow right in, leaving some nuclei and short alveoli behind as there described, till they make contact in the *centre*. There is still some uncertainty, perhaps, concerning the meeting and *central* closure, the spindles of one side joining up in some way with the spindles from the other; but a *central* junction is quite definite.

The Sierra Redwood is actually a favourable subject for the observation of alveolar formation. The ovule being often elliptical in section it is easy to

see that the forms of the alveoli are an expression of their positions. Those at the ends of the longer axis frequently meet as they grow in, and appear V-shaped in section, actually they are pyramidal; those at the sides can grow in directly to the centre as continuous tubes, actually in shape long prisms. Grading from vegetative to fertile alveoli is also shown nicely, especially opposite the pollen tube above the invagination. The first division in the completed alveolus may divide it evenly into two or into a smaller outer and a larger inner cell, those subsequently forming archegonia having a somewhat smaller outer cell, which will form the neck, although when first organized it may appear relatively surprisingly large—Fig. 6*e*. In very young stages it may be impossible to decide what any given alveolus will become. In the lower third or so of the gametophyte the tissue becomes more compact, and its alveolar origin may be obscured, but from below the pollen tube bulge up to the apex the alveolar appearance is retained right up to fertilization. The functional archegonia, less numerous than in *S. sempervirens,* tend to be less in a complex and more scattered than in that species. These features are shown in Fig. 6*g,* a longitudinal section of the invagination area shortly before the division of the body cell.

As in *S. sempervirens,* but not so erratically nor so frequently, intermediates between divided alveoli, jacket cells, irregular archegonia, and normal archegonia, occur, as well as archegonia not in contact with a pollen tube—Fig. 6*f*. The archegonial structure opposite the pollen tube here is obviously formed from a centripetally growing alveolus based on the spore membrane and tube, and is of the same type as that figured for *S. sempervirens* in Fig. 4*f*. In this case the first division cutting off a cell which should have formed the neck was followed by a second. Also here, in occasional archegonia, a second nucleus has been seen prior to fertilization. This may represent a ventral canal nucleus, but as the division was not met the fact is simply recorded.

The approximate dates of the essential phases are as follows. Spore enlargement, growth of the mature vacuolate gametophyte, and of the pollen tube, take from the first week in April to the third week, or later, in July. Alveolar formation stages take to about the end of the first week in August. Later cellular formation, maturation of the archegonium and body cell occupy up to approximately August 21. In Co. Wicklow, Ireland, at least, collections taken between August 21–26 regularly show stages which include division of the body cell, which takes place just before fertilization, mature archegonia, fertilization, and even early stages in proembryo formation—these later stages occurring very quickly. A transverse section just before fertilization is shown in Fig. 6*h*. It shows the compact nucellus, the firm gynospore membrane, the alveolar indications still visible at places in the gametophyte tissue, the mature archegonia (those closest to the male cells, and probably those about to be fertilized, with protruding apices), and the male cell with its very distinct zone of minute starch grains. At this stage, *Sequoia sempervirens* shows greater destruction of the nucellar tissue, thinner gynospore membrane, less definite gametophyte tissue arrangement, flush archegonia, and male cells (Fig. 4*e*) in

which a special zone is poorly distinct if present at all. The subsequent developments have been already described for fertilization and proembryo by Looby and Doyle (1937), and for embryogeny by Buchholz (1939a).

Conclusions.

Buchholz (1939c) has recently published a short paper on the generic segregation of the Sequoias. In this he tabulates numerous differences between them, and concludes that they cannot be retained as species of one genus. To these points others might be added, notably perhaps the difference in wood structure in typical specimens. Many of these points may be only such as are natural to different species, but the differences in proembryo and embryogeny are more important. When to these are added the further differences in development, outlined in this paper, in gynospore origin, in tapetum, in early prothallial growth and expansion, and in maturer cellular formation in the prothallus, it is clear that the two redwoods differ essentially in practically every phase of their life-history. In no other coniferous genus have such differences between species been recorded; on the contrary true species of any other genus show extremely close similarities in their development. The other differences noted by Buchholz (1939c) are thus given greater importance, and we, therefore, without hesitation agree with him that the Sierra Redwood, the Big Tree, commonly now known as *Sequoia gigantea,* can no longer be retained as a species of *Sequoia*, a generic title to which *Sequoia sempervirens* has priority claim. It would be expected then that it would revert to its former name of *Wellingtonia gigantea* Lindl., but Buchholz produces evidence in support of a claim that that generic title is invalid, and proposes a new generic name retaining the specific one—*Sequoidadendron giganteum.* We are not quite convinced that such a new generic title is necessary, and propose, until opportunity offers a decision by the International Committee of Nomenclature, to return to the use of the more familiar *Wellingtonia.*

In the earlier paper on *Sequoia* (Looby and Doyle, 1937) it is perhaps true, as Buchholz (1939b) comments, that greater emphasis was laid on similarities rather than on differences between the two plants here in question. Even at that time we felt that this return to a generic separation would be necessary, but hesitated to make that return on the basis of a proembryo difference only, and postponed consideration of the matter till a fuller account of the life-history was available, as it now is. Certain remarks in that paper on the relationships of genera and the possible importance of certain developmental features might now be modified. It is not, however, proposed now to enter at all into the inter-relationships within the Taxodiaceae, which is obviously a very heterogeneous collection of genera. With the completion of data on *Athrotaxis,* now in hands, such questions may receive fuller discussion.

The material used was collected in the estate of Viscount Powerscourt, Enniskerry, Co. Wicklow, to whom thanks are due for his kindness.

War economy necessitated the over-reduction of certain of the figures, notably those in 5 and 6.

Summary.

1. The origin of the gynospore and the development of the female gametophyte up to the fertilization stage are described for *Sequoia sempervirens* and *Wellingtonia gigantea.*

2. *Sequoia* is characterized by:—many gynospore mother cells; groups of variously formed spore tetrads from symmetrical tetrahedral groups, like pollen tetrads, to linear tetrads; a one-layered tapetum of short duration, formed mainly from non-functioning archesporial cells; unsymmetrical development of micropile closing cells; meristematic activity of the lower part of the nucellus carrying the developing spore region well up from the ovule base; downward boring of several gametophytes into this tissue, and their characteristic form when young; basal free nuclear and upper vacuolate zones in the gametophyte (as already described by other authors); development of alveoli in the vacuolate area in contrast to the free cell formation below (claimed by Arnoldi but denied by Lawson); and archegonial origin from peripheral alveoli (suggested by Arnoldi and denied by Lawson).

3. *Wellingtonia* is characterized by:—one gynospore mother cell; a single linear three-celled tetrad; a tapetum formed from surrounding nucellar tissue and becoming relatively massive and permanent; symmetrical micropile closure; normal upward extension of the vacuolate gametophyte, to which growth in nucellus and integument is adapted; no free nuclear area, however ill-defined, at the base; typical alveolar formation all round the gametophyte; alveolar origin of the archegonia very clear; a male cell with a dense zone of small starch grains around the nucleus.

4. The suggestion of Buchholz that the two types must be segregated in separate genera is sustained. His suggestion of a new generic title *Sequoiadendron* for *Sequoia gigantea* instead of *Wellingtonia* is temporarily not accepted. *Sequoia gigantea* (Lindl.) Decn., the Big Tree, the Sierra Redwood, becomes again *Wellingtonia gigantea* Lindl. *Sequoia sempervirens* (Lamb.) Endl., the Coast Redwood, the Redwood, retains this name.

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